26 November 2015
EMA/CHMP/842555/2015
Committee for Medicinal Products for Human Use (CHMP)

# Assessment report

Spectrila

International non-proprietary name: ASPARAGINASE

Procedure No. EMEA/H/C/002661/0000

## Note

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

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# List of abbreviations

ADA Anti-drug antibodies

AHA,L-AHA L-aspartic acid-β-hydroxamate

ALL Acute lymphoblastic leukaemia

AML Acute Myeloid Leukaemia

Ara-C 1-β-D-arabinofuranosylcytosine

AS Asparagine synthetase

ASN L-asparagine

asparaginase L-asparaginase

ASP, L-ASP L-Aspartic acid

AUC Area under the drug serum concentration versus time curve

AUC0-72 Area under the drug serum concentration versus time curve from 0 up to 72 h

AUD Area under data

b.w. Body weight

BLLQ Below Lower Limit of Quantification

BM Bone Marrow

BMF group Berlin (B), Frankfurt (F), Münster (M) group

BMP Bone Marrow Puncture

CI Confidence interval

Cl Chloride

Cmax Maximum drug serum concentration

CNS Central Nervous System

COMP Committee for Orphan Medicinal Products

CR Complete remission

CRP C-reactive protein

CSF Cerebospinal fluid

CSR Clinical Study Report

CTCAE Common Terminology Criteria for Adverse Events

CZE Capillary Zone Electrophoresis

Da Dalton

DCOG Dutch Children Oncology Group

DMC Data Monitoring Committee

DNA Desoxyribonucleic acid

DXM Dexamethasone

*E. coli* Escherichia coli

e.g. For example

FAS Full Analysis Set

GCP Good Clinical Practices

GLN, L-GLN L-glutamine

GLP Good laboratory practice

GLU, L-GLU L-glutamic acid

Gy The international standard unit of radiation dose

HPLC High pressure liquid chromatography

hr hour

HR High risk

i.a. Intraarterial

i.m. Intramuscular

i.p. Intraperitoneal

i.v Intravenous

IC50 Concentration that inhibits by 50 %

IM Intramuscularly

inj. Injection

ITT Intent-To-Treat

IU International Unit

K+ Potassium

Km Michaelis constant

L-ASN, D-ASN L-asparagine, D-asparagine

LBL Lymphoblastic lymphoma

LD10/50 Dose lethal to 10% or 50 % of treated animals

LLN Lower Limit of Normal

LLOQ Lower Limit of Quantification

MAA Marketing Authorisation Application

MAAT medac asparaginase activity test

MR Medium risk

MRD Minimal residual disease

mRNA Messenger ribonucleic acid

MSC Bone marrow mesenchymal stromal cell

MTX Methotrexate

n.i. Not indicated

Na+ Sodium

NH3 Ammonia

NHL Non-Hodgkin Lymphoma

NOAEL No observed adverse effect level

NOD Non-obese diabetes

NOEL No observed effect level

p Possibility

p.o. Per os (oral)

p.v. Paravenous

PD Pharmacodynamics

PEG Pegylated

P-gp P-glycoprotein

pH Negative logarithm of H+ concentration

PK Pharmcokinetics

PP Per Protocol

recombinant asparaginase Recombinant L-asparaginase

RP-HPLC Reverse phase high pressure liquid chromatography

s.c. Subcutaneous

SA Scientific Advice

SAP Statistical Analysis Plan

SCID Severe combined immunodeficient

SCT Stem Cell Transplantation

SD Standard deviation

SR Standard risk

T/C Treated to control values (in percent)

t½ Elimination half-life

tmax Time of occurrence of Cmax

U Unit(s)

U/L Units per litre

| | |
|---|---|
| ULN | Upper Limit of Normal |
| UV | Ultra violet light |
| VCR | Vincristine |

# 1. Background information on the procedure

## 1.1. Submission of the dossier

The applicant medac Gesellschaft fuer klinische Spezialpraeparate mbH submitted on 5 December 2013 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Spectrila, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 17 November 2011.

Spectrila was designated as an orphan medicinal product (EU/3/04/258) on 26 January 2005 in the following indication: Treatment of acute lymphoblastic leukaemia. The orphan designation was withdrawn by the applicant on 10 December 2015.

The applicant applied for the following indication: Spectrila is indicated as a component of a curative antineoplastic combination therapy for infants, children, adolescents and adults with acute B/T cell lymphoblastic leukaemia (ALL) or B/T cell lymphoblastic lymphoma (LBL).

**The legal basis for this application refers to:**

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that asparaginase was considered to be a known active substance.

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

***Information on Paediatric requirements***

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0084/2013 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0084/2013 was completed.

The PDCO issued an opinion on compliance for the PIP P/0084/2013.

***Information relating to orphan market exclusivity***

***Similarity***

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

***Protocol Assistance***

The applicant received Protocol Assistance from the CHMP on 15 June 2005. The Protocol Assistance pertained to clinical aspects of the dossier.

***Licensing status***

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

## 1.2. Steps taken for the assessment of the product

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

Rapporteur: Robert James Hemmings   Co-Rapporteur: Daniela Melchiorri

- The application was received by the EMA on 5 December 2013.
- The procedure started on 26 December 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 14 March 2014. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 15 March 2014.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 10 April 2014.
- During the meeting on21-25 April 2014, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. .
- The applicant submitted the responses to the CHMP consolidated List of Questions on 19 March 2015.
- The following GCP inspections were requested by the CHMP and their outcome taken into consideration as part of the Safety and Efficacy assessment of the product:
  - GCP inspections at one bioanalytical laboratory in Germany, one clinical investigator site in the Netherlands and the sponsor site in Germany have been conducted between July and August 2014. The integrated inspection report of the inspections carried out was issued on 5 November 2014
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 April 2015.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 7 May 2015.
- During the CHMP meeting on 18-21 May 2015, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 14 August 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 2 September 2015.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 10 September 2015.
- During the CHMP meeting on 21-24 September 2015, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the CHMP meeting on 21-24 September 2015, the CHMP agreed on a 2nd list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on 19 October 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the 2nd List of Outstanding Issues to all CHMP members on 6 November 2015.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 6 November 2015.
- The CHMP adopted through written procedure on 26 November 2015, in the light of the overall data

submitted and the scientific discussion within the Committee, a positive opinion for granting a Marketing Authorisation to Spectrila.

- The CHMP adopted a report on similarity of Spectrila with Atriance, Evoltra, Iclusig, Sprycel, Xaluprine and Blincyto on 26 November 2015

# 2. Scientific discussion

## *2.1. Introduction*

**Problem statement**

Acute lymphoblastic leukaemia (ALL) is a heterogeneous group of lymphoid neoplasms that result from monoclonal proliferation and accumulation of lymphoblasts in the bone marrow, peripheral blood and other organs.

ALL is the most commonly diagnosed cancer in children and represents 25% of cancer diagnoses among children younger than 15 years. In absolute terms, however, ALL is a very rare disease, even in children. Its incidence has a bimodal distribution with a sharp peak among children aged 2 to 3 years (>90 per million per year), with rates decreasing to 30 per million by age 8 years
(NCI at the NIH: Childhood Acute Lymphoblastic Leukemia Treatment, March 2013). A second steady increase in the incidence of ALL begins at approximately 50 years of age, with a peak incidence of about 2 per 100,000. Overall, estimated incidence in the EU is 1.28 per 100,000 persons, corresponding to a total of approximately 5,600 new cases per year.

The recognised chromosomal abnormalities in ALL result in biological differences in the condition, and are important for prognosis and for therapeutic decisions. For example, Philadelphia chromosome positive (Ph+) ALL, which is caused by the translocation of the BCR and the ABL genes, is associated with a very poor prognosis.

ALL is divided into subtypes based on the presence of B- or T-cell lineage-specific differentiation antigens detected on the surface of leukaemic blast cells. Precursor B-cell ALL (including early pre-B and pre-B-cell ALL) is the most common (approximately 70% to 80%) subtype in both children and adults. Mature B-cell ALL (Burkitt's leukaemia / lymphoma) has been reported in 2% to 5% of children and adults. T-cell ALL has been diagnosed in 15% to 25% of paediatric and adult patients with ALL. It occurs more commonly in older adolescents and young adults than in young children, with an incidence of approximately 25% of all ALL in patients 16 to 21 years of age.

The treatment for ALL typically consists of a remission-induction phase, an intensification (or consolidation) phase and continuation / maintenance therapy to eliminate residual disease. Treatment is also directed to the Central Nervous System (CNS) early in the clinical course to prevent relapse attributable to leukaemic cells sequestered in this site (Pui et al, 2008).

Treatment depends on the use of intensive multi-agent chemotherapy given over 6 to 9 months in combination with central nervous system prophylactic therapy with cranial radiation and intrathecal chemotherapy followed by maintenance chemotherapy for 2 to 3 years.

All phases of treatment involve combination chemotherapy. Current treatment guidelines (European LeukemiaNet and National Comprehensive Cancer Network) recommend treatment with Tyrosine Kinase Inhibitors (TKIs). In Europe, the following TKIs are currently approved for the treatment of ALL and LBL: Glivec (imatinib); Sprycel (dasatinib); Tasigna (nilotinib); Iclusig (ponatinib).

In addition, drugs used during induction typically include vincristine, prednisone, cyclophosphamide, doxorubicin and L-asparaginase. Cytarabine and methotrexate are often added during consolidation / intensification. Maintenance therapy frequently includes 6-mercaptopurine, methotrexate, steroids and vincristine (NCCN guideline, November 2015).

Allogeneic blood stem cell transplantation is of great importance especially in patients at high risk or after a recurrence.

All these protocols involve the use of L-asparaginase during induction and consolidation /intensification phases. The objective of asparaginase therapy is to deplete physiological asparagine so that it is unavailable to tumour cells.

Historically, native E coli-derived asparaginase was used as the initial intervention. However, treatment-limiting immune response can occur. Once hypersensitivity is apparent, it is necessary to switch to a different asparaginase preparation. Therapy can then continue until a treatment-limiting immune response to the substituted asparaginase occurs.

Excellent progress has been made in the therapy of childhood ALL over the past five decades with combination chemotherapy and central nervous system (CNS) prophylaxis having improved the cure rate from 5% in 1950 to 85% in 2000 (Bailey et al, 2008). In contrast, while dose-intense multi-agent regimens administered to adult patients with ALL now achieve remission rates exceeding 80%, 5-year survival is only around 40% (Faderl et al, 2008).

**About the product**

Spectrila is a recombinant L-asparaginase produced in E. coli. The amino acid sequence and enzymatic activity of the recombinant asparaginase has been developed to be identical to Asparaginase medac which is an asparaginase preparation derived from a natural E.coli mutant.

Asparaginase hydrolyses asparagine to aspartic acid and ammonia.  In contrast to normal cells, lymphoblastic tumour cells have a very limited capacity for synthesising asparagine because of a significantly reduced expression of asparagine synthetase. Therefore, they require asparagine which diffuses from the extracellular environment. As a result of asparaginase-induced asparagine depletion in serum, protein synthesis in lymphoblastic tumour cells is disturbed while sparing most normal cells. Asparaginase may also be toxic to normal cells that divide rapidly and are dependent to some degree on exogenous asparagine supply.

Due to the asparagine concentration gradient between the extra- and intravascular space, asparagine levels are subsequently also reduced in the extravascular spaces, e.g. the cerebrospinal fluid. (see SmPC section 5.1).

The applicant claimed the following indication:

Spectrila 10,000 U is indicated as a component of a curative antineoplastic combination therapy for infants, children, adolescents and adults with acute B/T cell lymphoblastic leukaemia or B/T cell lymphoblastic lymphoma.

The CHMP recommended the following indication:

Spectrila is indicated as a component of antineoplastic combination therapy for the treatment of acute lymphoblastic leukaemia (ALL) in paediatric patients from birth to 18 years and adults. (see SmPC section 4.1).

Spectrila should be prescribed and administered by physicians and health care personnel experienced in the use of antineoplastic products. It should only be given in a hospital setting where appropriate resuscitation equipment is available.

Spectrila is usually employed as part of combination chemotherapy protocols with other antineoplastic agents (see also section 4.5 of SmPC).

The recommended intravenous dose of asparaginase is 5,000 units per square metre (U/m²) body surface area (BSA) given every third day.

Treatment may be monitored based on the trough serum asparaginase activity measured three days after administration of Spectrila. If asparaginase activity values fail to reach target levels, a switch to a different asparaginase preparation could be considered (see section 4.4).

The daily amount of Spectrila needed per patient can be diluted in a final volume of 50 – 250 ml sodium chloride 9 mg/ml (0.9 %) solution for injection. The diluted solution of asparaginase may be infused over 0.5 to 2 hours. Asparaginase must not be administered as a bolus dose (see SmPC section 4.2).

## *2.2. Quality aspects*

### 2.2.1. Active Substance

***General information***

Asparaginase is an enzyme obtained from *Escherichia coli* (*E. coli*) which converts asparaginase to aspartic acid and ammonia. The active substance, recombinant asparaginase, differs from wild type (wt) asparaginase at four amino acid positions. The active substance asparaginase (asparaginase aminohydrolase) appears as white crystals and is freely soluble in water. It is active as a homotetramer, with a total molecular mass of 138,368 Da (approximately 35 kDa per monomer).

***Manufacture, characterisation and process controls***

The active substance is manufactured according to standard techniques for the bulk production of a recombinant protein/medicinal product from *E. coli* using a plasmid expression system with a MCB and WCB. The plasmid contains an engineered version of the wt gene leading to a protein with 4 amino acid substitutions. Induction occurs by elevating the temperature which inactivates the repressor protein. The pre-asparaginase produced (with signal peptide) is secreted by a cellular translocation mechanism during which the signal peptide is cleaved post-translationally yielding mature asparaginase in the periplasm of the cells. One vial of the WCB is used for the pre-fermentation culture which is then used to inoculate the production culture. This is then followed by harvesting, homogenisation and filtration prior to purification using sequential chromatography steps.

***Description of Manufacturing Process***

In summary; a pre-culture is developed from a vial of the WCB for the inoculation of the production fermenter. The product is harvested by homogenisation of the cells and purified in three chromatographic steps before being conditioned and filtered (0.2 µm). A batch comprises the protein purified from a single harvest derived from fermentation of one vial of the WCB. The culture broth is processed so that the batch size consists of approximately 480 g of purified bulk active substance.

***Description of upstream processing and fermentation***

A frozen cryovial of the WCB is allowed to thaw at room temperature and the cells are then resuspended by mixing. The contents of the cryovial are used to inoculate a pre-culture fermenter containing fermentation medium which is qualitatively the same as that used for production fermentation. During pre-culture fermentation, the cells are cultured under defined conditions. The pre-culture cell suspension is then harvested and used to inoculate the production fermenter.

Following inoculation of the production fermenter, the cells are cultured under defined conditions in two steps. In the first step (up to nutrient limitation), the cells multiply, while in the second step μ-control (additional nutrient feed) is switched on to enable high cell density fermentation.

The cells are allowed to grow in the fermenter at a defined temperature for a fixed time range, after which time production of asparaginase is induced by a temperature shift. The fermentation process is terminated by gradually reducing the temperature.

***Description of downstream processing and purification***

The fermentation broth is transferred to the downstream processing suite and processed further. Homogenisation is performed in three cycles.

After sampling, the homogenate is subjected to tangential flow. Microfiltration is performed in three steps:

1. diafiltration (1) against phosphate buffer,

2. concentration, and

3. diafiltration (2) against phosphate buffer.

The microfiltration permeate containing the active substance is concentrated. The ultrafiltration retentate is then diafiltered against phosphate buffer and subsequently diluted. Clarification steps are performed prior to loading the first chromatography step. AEC (anion exchange chromatography) is performed using a liquid chromatography system at room temperature. Following equilibration, the column is loaded with the AEC-loading material and unbound proteins and impurities are subsequently washed from the column. Elution is performed and the AEC main fraction is collected. CEC is performed in two separate cycles using a liquid chromatography system at room temperature. In each cycle, half of the AEC main fraction is used as the starting material. Following equilibration, the column is loaded with the conditioned AEC main fraction and unbound proteins and impurities are subsequently washed from the column. IPCs tests are performed separately on the two CEC main fractions. Like CEC, HIC is performed in two separate cycles using a liquid chromatography system at room temperature. The starting material for each HIC cycle is one of the two CEC main fractions. Following equilibration, the column is loaded with the conditioned CEC main fraction and unbound proteins and impurities are subsequently washed from the column. The pooled HIC main fraction containing the active substance is recirculated from the diafiltration vessel through an ultrafiltration unit.

Conditioning of the bulk active substance solution is performed in three steps:

1. Diafiltration (1) against WFI.

2. Concentration to the target final weight.

3. Diafiltration (2) against WFI.

Following conditioning, the bulk active substance solution is pumped through a filtration unit.

The filtered active substance is collected in a sterile bag and stored at 2 – 8°C until being shipped to the finished product manufacturer. Prior to storage, samples of the filtered drug substance solution are taken for release testing.

**Characterisation**

Characterisation focused on analysis of the minor peaks around the main peaks from SEC and RP-HPLC analysis. Peaks additional to the main peak have been categorised as product-related substances or product-related impurities and are acceptably characterised. The product-related substances consist of pre-peaks and post main peaks (RP-HPLC), 'group 1' and 'group 2' substances respectively. The activity

of these peaks has been shown to be almost indistinguishable to the main tetrameric fraction. No truncation variants have been identified. Product related impurities are defined as the aggregates and monomer identified by SEC. Earlier development batches have been characterised for charge isoform variants; however the Applicant had not fully developed and validated a suitable method at the time of initial submission. Capillary zone electrophoresis (CZE) is the favoured method which will be introduced. As this is stability indicating the Applicant has been requested to introduce the validated method prior to approval. This has been confirmed and the Applicant has validated the method for both active substance and finished product. In addition, acidic and alkaline variants were characterised, particularly in terms of impact on efficacy and safety. A characterisation report has been subsequently provided. The two isoform species identified are tetrametic in nature (acidic and alkaline components) and correlate well with species detected by AEC (which correlate with the RP-HPLC species). Please see stability of active substance and finished product discussions below. The enzyme activity of these species are sufficiently similar to the activity of the main isoform variant. No effect on efficacy and safety is expected. Process-related impurities (DNA/HCP/Endotoxin) are routinely maintained at acceptable levels.

**Control of critical steps and intermediates**

Four categories of IPCs are used to monitor, adjust and control process performance at each stage of the manufacturing process. Set points, technical controls and manufacturing controls mostly represent physical parameters which the Applicant stated can only vary within predefined limits controlled by largely automated equipment (temperature, time, pH, glucose, oxygen, pressure, volume, flow rate etc). The parameters and ranges of IPCs (in-process controls) are considered acceptable and appear suitable for control of the upstream and downstream processes. It was initially considered that a number of the IPCs were wide, as specifications had been set based on pilot and commercial scale batches, however the affected IPCs have been satisfactorily tightened.

**Process validation**

Process validation was conducted using the three full-scale batches. IPCs for all upstream and downstream processing steps and release data are in good agreement demonstrating the capacity to manufacture a consistent active substance. Evidence for suitable validation of the microfiltration/ultrafiltration filter, column chromatography lifetime, cleaning validation and active substance transport validation for the full scale process have also been provided. The holding time specifications for the micro/ultrafiltration retentate have been validated. The commercial-scale manufacturing process is suitably validated.

**Manufacturing process development and comparability**

There have been extensive changes to the active substance manufacturing process since early development, which have been divided into processes I-V, where V is 'pilot scale' and then up-scaling from pilot scale to 'commercial scale A'. The most significant changes were the introduction of a new antibiotic free MCB and significant up-scaling of the process. No commercial scale or 'pilot scale' batches have been tested in clinical trials (only earlier development batches from process II and III). So comparability was required to be demonstrated between the commercial-scale process and the material used in clinical trials. The initial finding of comparability by the applicant was not fully supported and this was a concern raised during the procedure. The Applicant has since provided a more comprehensive comparability assessment, to address the concerns raised. The differences in CZE, AEC and MS/MS parameters were generally related to the age of the early batches tested (isoform degradation products are clearly evident from stability studies) or the way the samples were treated in the case of the MS/MS data. Comparability at the level of active substance has been acceptably demonstrated.

***Specification***

The proposed release tests are acceptable, including measures of enzyme activity, and methods to control for product-related substances and product related impurities (aggregates). Several specifications have been tightened nevertheless, it was considered that further tightening could be made some specifications. The specifications have been tightened. As the specification is based on limited commercial-scale batches the Applicant is recommended to review the specification when more clinically tested batches are produced and to revise the specification with a post-approval variation as appropriate.

***Reference standard***

No compendial reference standard is available for r-L-asparaginase. During evaluation a suitable standard for the active substance and finished product was introduced.

***Stability***

Data was only available for one batch at the recommended time-point, therefore further data to support the initial shelf-life claim was requested. Taking into account that all other parameters were within specifications and there is evidence that the levels of main peak and isoform species are clinically qualified with no likely impact on efficacy and safety, an appropriate shelf-life was accepted based on the data provided at the time of the Opinion.

## 2.2.2. Finished Medicinal Product

***Description of the product and pharmaceutical development***

***Composition of the product***

The finished product is a sterile, lyophilised powder for concentrate for solution for infusion containing 10,000 U of the active substance asparaginase. The finished product contains sucrose as excipient. After reconstitution with water for injections the preparation is administered by intravenous infusion as indicated in the SmPC.

The container of the product consists of a colourless glass vial, a rubber stopper and a safety aluminium cap.

***Process Development***

Four lots of finished product have been manufactured at pilot scale for clinical trials. The changes that took place between these lots were changes in the active substance manufacturing process, introduction of a new manufacturer for the finished product and removal of citrate buffer from the initial finished product formulation. It has been clarified that the lyophilisation time for these two clinical trial batches was also equivalent. Comparability of the finished product was sufficiently demonstrated for these batches.

However, following clinical trials the process has seen a change in MCB (antibiotic free) and up-scaling of the active substance/ finished product manufacturing processes to commercial scale. Only one full scale finished product commercial batch had been manufactured to GMP at submission (two others were non-GMP) which was not tested in clinical trials, therefore the Applicant conducted a comparability assessment, based on analysis of characterisation, IPC and release data. The statement of comparability was not fully supported as there were differences in the CZE profile of the finished product identified during characterisation and also a number of differences in the release data, which had not been suitably discussed, particularly in terms of expected impact on clinical outcome. The Applicant has subsequently submitted a more comprehensive comparability report which addresses the differences observed.

Sufficient comparability has been demonstrated as it was concluded that the differences seen are relatively minor and also not considered to impact on clinical efficacy and safety.

***Manufacture of the product and process controls***

Manufacture consists of formulation (with sucrose and water for injections (WFI)) filtration (bioburden reduction and sterile filtration), aseptic filling and lyophilisation steps. There were some issues raised concerning the control of the process, (wide acceptance criteria in some cases, for example osmolarity) however these issues were adequately resolved. There was considerable variability seen in the pH for the three commercial-scale batches which the applicant was requested to address. In their response, the Applicant stated that the pH electrode may not be the most appropriate to use. A new system has been implemented and the Applicant has subsequently tightened the specification for pH which is considered acceptable.

The 'commercial scale' process was validated through manufacture of three successive validation batches. All validation issues have been considered suitably resolved.

***Product specification***

The key finished product release tests are based on those used to characterise the finished product. The release tests are acceptable; however there were several issues which required clarification. The Applicant was requested to set release specifications which more suitably reflected the commercial scale batches for RP-HPLC for the finished product. Also the need for shelf-life specifications was questioned for the main peak. The Applicant has tightened the specifications and also removed the shelf-life specification. In addition, the specification for HPSEC was requested to be tightened and the shelf-life specification was not justified by stability data. The Applicant has tightened the specification and the shelf-life specification has been removed. It appeared that the proposed release specifications for CZE were not clinically qualified. The Applicant has set release specifications for CZE based on the commercial manufacturing process and it can be concluded that the CZE specification is clinically qualified. The specifications can be accepted. Batches used in the clinic were retrospectively tested by CZE and the commercial-scale batches were within a sufficiently similar range, despite the age of the clinical batches. The stability of these old batches was also confirmed by AEC and RP-HPLC which were highly comparable to newly manufactured commercial batches. The Applicant was recommended to revisit some specifications when more commercial batch data for this new method becomes available and to submit a post-approval variation as appropriate.

***Stability of the product***

A significant concern during the procedure regarding stability of the finished product was raised, as there was a possible minor downward trend in the main peak as detected by CZE with a concomitant increase in alkaline isoform species. As limited stability and characterisation data were available it could not be determined whether this decrease would have an impact on efficacy and safety. The Applicant has provided further data and requested a shelf-life of 30 months. Real-time data is available for 2 commercial scale batches at 24 months. The data do further confirm a minor decrease in the main peak/increase in alkaline isoform. However, the enzyme activity of the isoform species has been shown to be sufficiently similar to the main peak, so no effect on efficacy and safety are expected. Batches used in the clinic and commercial batches were comparable and the values at 24 months are sufficiently similar to the retrospective analysis of clinical trial batches. All other measurement parameters showed no trends. Therefore this concern was considered resolved and a shelf-life of 24 months (2-8°C) can be accepted. However, as the stability data is limited, the Applicant is recommended to place the first three commercial batches following approval of the MAA on stability and to update the stability testing program to be in-line with ICH Q5C.

**Adventitious agents**

The product presents minimal risk from adventitious agent contamination. *E. coli* do not harbour mammalian viruses. There is suitable control of raw and starting materials, including characterisation of the cell bank, and no materials of animal or human origin are used in the manufacturing process. The production process contains several filtration steps to reduce any potential contamination and also includes in-process controls and release tests for the detection of adventitious agents, including endotoxins and bioburden. The active substance and finished product manufacturers also have GMP-certified hygiene measures in place. The adventitious agent safety evaluation presented is sufficient and adequate.

### 2.2.3. Discussion on chemical, pharmaceutical and biological aspects

There were three Quality significant concerns raised in the initial assessment in relation to the clarification of different active substance/finished product manufacturing 'scales'; comparability between batches used during clinical studies and the commercial manufacturing process and stability of the finished product. The Applicant provided adequate justifications and additional data and therefore these concerns were considered adequately resolved.

The first three commercial batches following approval of the MAA should be placed on stability and the stability testing program should be updated to be in-line with ICH Q5C. Some release specifications when more commercial scale batches used in the clinic are available should be revised.

### 2.2.4. Conclusions on the chemical, pharmaceutical and biological aspects

The active substance and the finished product have been appropriately characterised and satisfactory documentation has been provided. The results indicate that the active substance as well as the finished product can be reproducibly manufactured.

### 2.2.5. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended some additional points for further investigation.

## *2.3. Non-clinical aspects*

### 2.3.1. Introduction

Recombinant asparaginase (ASNase) was compared with the marketed reference preparation Asparaginase 10,000 E Medac in several pharmacodynamics (PD), pharmacokinetic and toxicity studies (including toxicokinetics and evaluation of the immunogenic potential). In addition, non-comparative studies to evaluate the effects of different dose-schedule on in vivo PD, safety pharmacology aspects, and local tolerance were carried out with rASNase. As different batches and formulations of rASNase have been used in non-clinical studies, a summary is provided in the table below.

**Table 1: Summary of rASNase preparations used for preclinical testing**

| Batch No. | Description | Type of Study | Study No. | GLP |
|---|---|---|---|---|
| F1AS9917/1/SF/P4 | Sterile drug substance solution | • *In vivo* efficacy study | MV 5893<br>MV 5907 | Non |
| M01006AA (A),<br>M00509AC (B),<br>M01006AA, (C) | Lyophilised sterile drug substance | • *In vivo* efficacy study | MV 6105 | Non |
| AnsB preparations | AnsB medac aggregates<br>AnsB medac tetramers<br>AnsB Pro Thera Bulk-Agent, | • *In vivo* efficacy study<br>• Comparative PK study in mice | MV 6838<br>MV 6839 | Non<br>Non |
| MC0201 | Lyophilised sterile drug substance (without additives) | Comparative PK study in dog (incl. amminoacid determination) | LPT 13106/2/01 | Yes |
| MC1102 | Sterile drug substance solution (without additives) | • *In vitro* enzymatic/kinetic study<br>• *In vitro* anti-proliferation study<br>• Safety Pharmacology studies<br>• Single dose Tox. study in rat<br>• 4-week Tox. study in rat (incl. determination of ADA)<br>• 4-week Tox. study in dog (incl. determination of ADA) | Röhm/Marburg<br>Cell Trend 05/03<br>LPT 16369/02 to<br>LPT 16373/02<br>LPT 16366/02<br>LPT 16367/1/02<br>LPT 16368/1/02 | Non<br>Non<br>Yes<br>Yes<br>Yes<br>Yes |
| MC0703 | Lyophilised drug product; formulation developed by medac; pilot scale batch preparation used for preclinical investigations; identical to the preparation used for clinical studies (MC1003) | • *In vitro* anti-proliferation study<br>• *In vivo* efficacy studies<br>• Comparative PK study in mice<br>• Comparative PK study in dog<br>• Local Tolerance test in rat | Cell Trend 11/03<br>MV 6773<br>MV 6839<br>LPT 16750/03<br>LPT 17237/03 | Non<br>Non<br>Non<br>Yes<br>Yes |
| MC1003 | Lyophilised drug product; formulation developed by medac norm scale production; used for clinical studies (MC-ASP.2/RHN) (MC-ASP.4/ALL) | • *In vivo* efficacy study<br>• Comparative PK study in dog | MV 7001<br>LPT 19030/05 | Non<br>Yes |
| MC1005 | Lyophilised drug product; new formulation without citrate buffer; used for preclinical and clinical studies (MC-ASP.5/ALL) | • Comparative PK study in dog<br>• Local Tolerance test in rat | LPT 19030/05<br>LPT 20153/06 | Yes<br>Yes |

## 2.3.2. Pharmacology

***Primary pharmacodynamic studies***

A number of publications from 1953 to 2006 describing the pharmacodynamic activity of various forms of L-asparaginase were submitted. The Applicant also provided reports of 8 non-clinical studies summarising the results obtained with different versions of r-L-asparaginase. A summary of available data is provided below.

The kinetic properties of the conventionally purified preparation of ASNase isoenzyme II from wild-type *E. coli* (Asparaginase 10000 E medac) and rASNase (MC1102) have been compared with special emphasis on the substrate specificities of the two preparations. No significant differences in the kinetic parameters of both ASNase preparations were detected.

The estimated Km values of 0.49 ± 0.05 mM for L-AHA and 3.9 ± 0.4 mM for L-GLN were in agreement with published data [Campbell, 1969; Miller, 1969b]. There were no significant differences between the pH-activity profiles of both ASNase preparations with any of the three substrates (L-ASN, L-AHA and L-GLN).

*In vitro studies investigating anti-neoplastic activity*

The anti-proliferative activities of Asparaginase 10000 E medac and rASNase batches MC1102 and MC0703, at 0.001 – 100 U/mL, have been compared *in vitro* using two different ASNase-sensitive human cell lines in two separate studies. Asparaginase 10000 E medac and rASNase both showed a dose-dependent inhibition of cell proliferation in the two cell lines tested compared to control.

*In vitro studies described in the literature*

*In vitro* studies analysed the cytotoxic effect of ASNase on isolated blood cells from patients with leukaemia and revealed that this effect is dependent on dose and sensitivity of cells against ASNase [Schreck, 1967; Schreck 1971].

Subsequently, the effect of native ASNase on the *in vitro* survival of bone marrow mononuclear cells obtained from previously untreated patients with ALL was described by Asselin and coworkers (1989). The *in vitro* killing of leukaemia cells was independent of concentration from 0.0001 to 0.1 U/ml (P = 0.3 to 0.7), suggesting the absence of a dose response at ASNase concentrations tested. Addition of these lower ASNase doses markedly decreased the L-ASN concentration in culture medium [Asselin B et al., 1989].

Guo Q-L et al. described the establishment of a recombinant ASNase from a system (PKA/CPU210009) that displayed extremely high expression efficiency for this enzyme [*Guo, 2002*]. Both, *in vitro* and *in vivo* studies which were performed by this group of investigators, confirmed that the antineoplastic activity of the recombinant ASNase, was as effective as wild-type ASNase [Guo, 2002].

*In vivo studies*

In a murine model of leukaemia*,* the anti-leukaemic activity of rASNase (Batch F1AS9917/1/SF/P4) was tested and compared to that of Asparaginase 10000 E medac. Cyclophosphamide was used as reference compound [Report MV5893, 2000]. Neither Asparaginase 10000 E medac nor rASNase induced a significant change in survival time when compared to control.

*Effects on human leukaemia xenografts in SCID mice:* The ALL-SC19 model was used to compare the antileukaemic activity of rASNase (Batch F1AS9917/1/SF/P4) and Asparaginase 10000 E medac to that of the reference compound vincristine [Report MV5907, 2000]. Tumours of mice treated with rASNase developed in a similar manner to those in control mice. Tumours of mice treated with Asparaginase 10000 E medac or vincristine disappeared within the treatment period and did not regrow by Day 50. Asparaginase 10000 E medac and vincristine caused a distinct reduction in body weight.

Further investigations were performed in order to clarify the reason for the observed results. The anti-leukaemic activities of three lyophilised ASNase preparations (rASNase containing aspartate, ASNase 10000 E medac, and rASNase without aspartate) were evaluated using the human acute lymphatic leukaemia (ALL-SCID-19) model in mice (strain NOD/SCID) and compared to the reference compound, vincristine. At 4,000 U/kg, an equivalent long-standing anti-leukaemic effect was observed with all ASNase preparations. At that dose, ASNase preparations were significantly more effective than

the reference compound, vincristine when administered at the maximum tolerated dose. The delay of leukaemia development was significantly increased at 4,000 U/kg ASNase when compared to that observed with vincristine. At 1,000 U/kg, Asparaginase 10000 E medac (100 % complete remission with regrowth from Day 35) was significantly superior to rASNase regarding the delay of development of leukaemic nodules. All preparations were equally well tolerated and induced moderate, dose-dependent reductions in body weight without lethality. The stabiliser, aspartate had no influence on the anti-leukaemic activity of the final lyophilised product, rASNase.

A third study was performed in order to elucidate whether any differences in anti-leukaemic activity exist between Asparaginase 10000 E medac and rASNase [Report MV6773, 2003]. Control animals were treated with water/saline and positive control animals were treated with vincristine (1 mg/kg i.p. on Day 13). Asparaginase 10000 E medac led to a dose-dependent inhibition of tumour growth. At 8,000 U/kg/day, all mice were tumour-free by Day 36; then regrowth was observed. At 500 U/kg/day an optimum T/C value of 8 % was observed with 1/10 complete remissions. Recombinant ASNase (Batch MC0703) revealed significantly lower anti-leukaemic activity at equal dose levels compared to Asparaginase 10000 E medac. Treatment with rASNase showed significant T/C values between 41% (at 2000U/kg) and 7% at 8000U/kg and 500U/kg was completely inefficient. A 16-fold higher dose of MC0703 (8,000 U/kg/day) was equally effective with 500 U/kg/day Asparaginase 10000 E medac. The two ASNase preparations were well tolerated with mild body weight loss observed in both groups.

In order to assess the impact and the hypothesis that different aggregates which are sporadically present in the clinical preparation of Asparaginase 10000 E medac could be responsible for different pharmacological effects, the anti-leukaemic activity of five different ASNase preparations were tested with the aid of the ALL-SCID 19 model in mice [Report MV6838, 2004]. According to their anti-leukaemic activity (high activity to low activity), the test preparations were ranked: Isolated high molecular weight aggregates from Asparaginase 10000 E medac > Asparaginase 10000 E medac> tetramers = rASNase = bulk agent. All preparations led to a significant, dose-dependent inhibition of leukaemia growth. Aggregates resulted in complete remissions at 1,000 or 8,000 U/kg/day lasting until Day 25 or 35, respectively. The anti-leukaemic activity was comparable to those of Asparaginase 10000 E medac at a dose of 8000 U/kg/day and significantly better at the lower dose of 1000 U/kg/day. Both, Asparaginase 10000 E medac and aggregates caused a substantial number of complete remissions, but finally the tumours regrew. All preparations were well tolerated and were not associated with mortality.

Based on former investigations with rASNase, different schedules of rASNase administration have been compared in order to analyse whether anti-leukaemic efficacy is schedule-dependent. Daily administration for five days (one or two injections per day) was compared to treatment every other day for 4 days, which is the schedule used in the previous studies [Report MV7001, 2004]. The twice daily schedule resulted in the highest efficacy among the schedules tested: complete remissions were observed in 10/10 mice between Days 36 and 44, respectively. Tumour regrowth started on Day 47, but 6/10 animals were tumour-free at sacrifice on Day 64. Bolus injection of the same daily dose did not induce complete remissions and the mice had to be sacrificed with large tumours on Day 49. Treatment every two days caused complete remissions in 6/10 animals on Day 41, and 1/10 animals remained tumour-free by day 49. Vincristine led to transient complete remissions in 9/10 mice, but tumours regrew. All treatment schedules were well tolerated; however, body weight loss was recorded in all groups with the maximum body weight loss (- 14 %) observed in the most effective group. Sustained levels of ASNase between sequential injections seemed to be most effective. The twice daily treatment resulted in highest efficacy and caused 60 % tumour-free survivors.

A number of *in vivo* studies have been described in the literature, investigating different treatment schedules in mice [Vadlamudi, 1970]; [Sugiura, 1969]; investigating the sensitivity of seven paediatric acute lymphoblastic leukaemia xenotransplanted into immunodeficient NOD/SCID mice [Fichtner, 2003]; the anti-tumour effect of a recombinant ASNase in mice [Guo, 2002]; investigating guinea pig serum and

ASNase from E. coli in various tumour models and species [Kidd,1953; Charles, 1981]; [Hardy, 1970]; [MacEwen EG, 1992]; [Simon, 2006].

***Secondary pharmacodynamic studies***

No studies were submitted on secondary pharmacological effects of r-L-asparaginase which was considered acceptable by the CHMP (see discussion on non-clinical aspects).

***Safety pharmacology programme***

**Table 2: Safety pharmacology studies conducted with recombinant asparaginase**

| **Type of study (species)** | **Study No.** | **Gender and No. per Group** | **Dose (single) or concentration** | **Results** |
| --- | --- | --- | --- | --- |
| Cardiovascular and respiratory (dog) | LPT 16369/02 | 5 males | 1000 U/kg i.v. | 1,000 U/kg was considered to be the highest sub-toxic dose level. Recombinant ASNase had no effect on cardiovascular or respiratory parameters. There was no evidence of QT prolongation. |
| Effects on diuresis and saluresis (rat) | LPT 16370/02 | 10 females | 100, 1000, 10000 U/kg i.v. | A significant increase in urine excretion was noted at 10,000 U/kg by ~ 33 % (cumulative interval: 0-24 hours). Excretion of $Na^+$, $Cl^-$ and $K^+$ was increased at all doses. The effect was statistically significant for excretion of $Na^+$ at 10,000 U/kg and for excretion of $K^+$ and $Cl^-$ at all doses.<br>NOEL: < 100 U/kg |
| Effects on spontaneous motility (mouse) | LPT 16371/02 | 8 females | 100, 1000, 10000 U/kg i.v. | No effect on spontaneous motility was observed at up to 10000 U/kg.<br>NOEL: > 10,000 U/kg |
| Effects on hexobarbital sleeping time (mouse) | LPT 16372/02 | 8 females | 100, 1000, 10000 U/kg i.v. | No effects on hexobarbital sleeping time were observed at up to 10,000 U/kg.<br>NOEL: > 10,000 U/kg |
| Spasmolytic/ spasmogenic properties (guinea pig ileum) | LPT 16373/02 | 6 males | 5, 10, 25, 50 U/mL | No agonistic/spasmogenic effect up to the highest tested concentration was observed. No antagonistic/spasmolytic effect was observed against the agonist acetylcholine, histamine and barium chloride up to the highest concentration tested (50 U/mL). |

Effects on insulin release and glucose homeostasis have been described in numerous studies [Khan, 1969] [Lavine, 1980; Lavine 1984]; [Pou, 1991]; [Clausen, 1989].

Effects on the pancreas: Pancreatic toxicity occurs most likely due to an inhibition of insulin synthesis caused by reduced levels of amino acids ASN and probably GLN. About 5 % of human adults treated with ASNase develop overt pancreatitis that may be fatal. Fatal haemorrhagic pancreatitis after ASNase therapy was reported in one dog which was treated for multicentric malignant lymphoma with ASNase (12,000 U) [Hansen, 1983].

Effects on the liver: It is thought that one of the main reasons for ASNase toxicity is its glutaminase activity. L-glutamine is the main form of nitrogen transportation in blood, and prolonged depletion of this amino acid during asparaginase therapy causes serious biochemical disorders in the body, particularly in liver [Capizzi, 1970; Oettgen, 1970). A glutaminase-free asparaginase with potent anti-lymphoma activity isolated from Vibrio succinogenes was not hepatotoxic even after prolonged treatment [Durden, 1983].

Effects on coagulation: Several investigations show that ASNase treatment of acute lymphoblastic leukaemia patients causes severe deficiency in antithrombin with the consequence of increased risk of thrombosis. However, the mechanism responsible for the decrease of plasma antithrombin caused by ASNase remains elusive [Hernandez-Espinosa, 2006]. Coagulation disorders can occur due to the inhibition of protein synthesis caused by ASNase [Rogers, 1992].

***Pharmacodynamic drug interactions***

The potential for drug interactions following co-administration with methotrexate, 1-β-D-arabinofuranosylcytosine, dexamethasone and vincristine has been described.

*Co-administration with methotrexate (MTX)*

ASNase inhibits protein synthesis and cell replication, thus, it may interfere with the action of MTX which requires cell replication for its anti-neoplastic effect [Capizzi, 1974; Capizzi, 1981].

*Co-administration with cytosine arabinoside (1-β-D-arabinofuranosylcytosine; ara-C)*

A schedule-dependent effect of ara-C and ASNase was observed both *in vitro* and *in vivo*. A sub-additive therapeutic effect of simultaneous exposure of ASNase and ara-C was observed in the murine leukaemia cell line L5178Y/asn-. The observed synergy was thought to be related to the timing of ASNase treatment and the "optimal therapeutic effect" occurs when sequential ASNase is administered before the cells recover from the ara-C effect [Schwartz, 1982].

*Co-administration with dexamethasone*

The incidence of osteonecrosis is as high as 20% in children treated for ALL. It is assumed that glucocorticoids such as dexamethasone (DXM) are responsible for the induction of osteonecrosis. ASNase hastened the occurrence of osteonecrosis which was observed as early as 4 weeks [Yang, 2009]. In a mouse model of glucocorticoid-induced osteonecrosis, the frequency of osteonecrosis after 6 weeks of dexamethasone plus ASNase was comparable to that observed following 12 weeks of dexamethasone alone.

*Co-administration with vincristine (VCR)*

ASNase decreases hepatic clearance of VCR, resulting in prolonged half-life and increased toxicity [Rogers, 1989]. Experiments in dogs have shown that the toxicity of vincristine may be additive with that of ASNase if both agents are administered concomitantly [Rogers 1989; Northrup 2002].

The effects of L-ASNase on hepatic clearance/metabolism of VCR may also be responsible for the observed neurotoxicity when the two agents are co-administered [Martindale Monographs, 2002].

The combination of DEX with VCR and ASN resulted in a significantly higher exposure to DEX when compared to DEX alone as observed in a panel of childhood ALL continuous xenografts derived from primary patient biopsies (ALL-7 and ALL-19) [Szymanska, 2012].

## 2.3.3. Pharmacokinetics

The primary aim of the pharmacokinetic studies conducted was to evaluate the pharmacokinetics of rASNase preparations compared to that of the reference product, Asparaginase 10000 E medac. The preparations were generally administered as a single intravenous dose to mice and dogs. In addition, the effects of the ASNase on serum levels of L-asparagine (ASN) were determined. Analytical methods and pharmacokinetic studies conducted in dogs were performed in accordance with GLP.

**Table 3: Absorption studies conducted on behalf of the Applicant**

| Type of Study | Study No. | Species | Route of Admin | Dose | GLP-Status | Batch No. rASNase |
|---|---|---|---|---|---|---|
| *Pharmacokinetics (PK)* | | | | | | |
| Comparative PK study with 5 ASNase preparations in NOD/SCID mice (2004) | MV6839/LPT 17488 | Mouse | i.v. | Single | Non | AnsB medac aggregates, AnsB medac tetrameres, MC0703, AnsB Pro Thera Bulk-Agent, |
| Comparative pharmacokinetic evaluation of MC0201 and Asparaginase medac after single i.v. administration to Beagle (2002) | LPT 13106/2/01 | Dog | i.v. | Single | Yes | MC0201<br>Asparaginase 10000 E medac |
| Pharmacokinetics of MC0703 and Asparaginase 10.000 medac after single i.v. admin. to Beagle dogs (2004) | LPT 16750/03 | Dog | i.v. | Single | Yes | MC0703<br>Asparaginase 10000 E medac |
| Pharmacokinetics of rec. Asparaginase (MC1003 vs. MC1005) after single i.v. admin. to Beagle dogs (2006) | LPT 19030/05 | Dog | i.v. | Single | Yes | MC1003<br>MC1005 |

In study MV6839, the pharmacokinetic profile of 5 different ASNase preparations was determined following single i.v. injection in female mice (strain: NOD/SCID). Following the observed efficacy profile in the mouse, the hypothesis was that aggregate content in the preparations could influence the pharmacokinetic features and subsequently lead to different anti-leukaemic activity. Mice (n=27/group) received a single i.v. injection (2,000 U/kg) of the ASNase preparations. Serum was analysed by means of the $NH_3$ assay [LPT 17488/03] and interpreted with a non- and three-compartment model.

Isolated aggregates from Asparaginase 10000 E medac (58 % aggregates) and the clinically used Asparaginase 10000 E medac (20.5 % aggregates) had much longer retention times in mice ($t_{1/2}$= 5.54 and 3.17 hours, respectively) in comparison to the three remaining rASNase preparations with a proportion of only 0.4-1.0 % aggregates ($t_{1/2}$ = about 1.3 hours). In line with these findings, higher AUC and lower clearance values for high-aggregate content preparations (groups A 'Asparaginase Medac' and B 'Aggregates (Asparaginase Medac)') were observed when compared to low-aggregate content preparations (C 'tetramers (Asparaginase Medac)' – E 'rASNAse (bulk agent)'). Isolated aggregates from Asparaginase 10000 E medac showed a 4-fold increase in half-life of ASNase activity as well as a three-fold increased area under the curve (AUC) compared to isolated tetramers. Results of isolated tetramers from Asparaginase 10000 E medac were clearly comparable with results obtained with both batches of rASNase (groups D 'rASNase (MC0703)' and E 'rASNase (bulk agent)'). Furthermore, high AUC values and slow elimination clearly correlated with the highest anti-leukaemic activity as determined in the corresponding pharmacodynamic study previously described.

In study LPT 13106/2/01, the pharmacokinetic properties of rASNase (Batch MC0201) and Asparaginase 10000 E medac were compared in the beagle dog (n=3/group) following single i.v. administration at 1000U/kg. No mortality was observed during the 8-day follow-up/observation period. Once or repeated

vomiting was noted in all 3 animals treated with rASNase at 2 – 6 hours post-dose. Two of three animals vomited repeatedly between 2 and 6 hours after administration of Asparaginase 10000 E medac. Results from each assay are presented below.

$NH_3$ assay: At fifteen minutes post dose, rASNase or Asparaginase 10000 E medac resulted in a mean maximum ASNase serum activity ($C_{max}$) of 15,103 U/L and 23,022 U/L, respectively. These values decreased to a mean ASNase serum activity of 577 U/L and 905 U/L for rASNase and Asparaginase 10000 E medac, respectively at 96 hours post dose.

AHA assay: At fifteen minutes post dose, rASNase or Asparaginase 10000 E medac resulted in a mean maximum ASNase serum activity ($C_{max}$) of 14,939 U/L and 14,220 U/L, respectively. These values decreased to a mean ASNase serum activity of 619 U/L and 830 U/L for rASNase and Asparaginase 10000 E medac, respectively at 96 hours post dose.

MAAT assay: At fifteen minutes post dose, rASNase or Asparaginase 10000 E medac resulted in a mean maximum ASNase serum activity ($C_{max}$) of 12,282 U/L and 18,460 U/L, respectively. These values decreased to a mean ASNase serum activity of 490 U/L and 841 U/L for r-ASNase and Asparaginase 10000 E medac, respectively, 96 hours post-dose.

Overall, lower AUC values have been determined following rASNase when compared to Asparaginase 10000 E medac. $T_{½}$ was between 16.1 and 24.8 hours for the tested preparations. The amino acid serum levels were affected to a similar degree by the two ASNase preparations. Due to the remaining ASNase levels in serum 96 hours post-dose, no recovery of amino acids was possible until 8 days post-dose of the compounds.

Overall, depending on the ASNase activity assay used, there was a slight trend towards an increase in the AUC for the reference product (in terms of the mean values); however, taking the observed variability into account, overall the observed $C_{max}$ and AUC values were statistically similar.

In a follow up study, the pharmacokinetic properties of a different batch of rASNase (Batch MC0703) were compared to that of Asparaginase 10000E medac [LPT 16750/03]. Single i.v. doses of 250 or 1,000 U/kg were administered to dogs (n=5/group). Following intravenous administration of a single dose of ASNase, no mortality or no signs of toxicity were observed. $AUC_{0\text{-}168h}$ increased in a dose-related manner. Serum activity levels decreased with time and $t_{1/2}$ ranged from 18.36 to 20.71 hours. There were no differences in the pharmacokinetic parameters for both preparations tested.

In a separate study, the pharmacokinetic properties of two lyophilised rASNase preparations (MC1003 and MC1005) were compared in dogs following single i.v. administration [report LPT 19030/05 and IKP study no.: 077/05-055.AE]. The rASNase MC1005 formulation is a lyophilised drug product without citrate buffer. Animals (n=5/group) were given rASNase at 1,000 U/kg via slow intravenous injection. Administration of MC1003 or MC1005 resulted in a comparable $AUC_{0\text{-}168}$ and $C_{max}$ of activity (measured via the AHA assay). No statistical difference was observed between the lyophilised rASNase preparations MC1003 and MC1005; hence exposures were similar irrespective of citrate content/inclusion.

Asparaginase is distributed mainly within the intravascular space, with minimal blood-brain barrier penetration. Therefore, ASN depletion by ASNase mainly occurs within the intravascular space. Since this builds up a concentration gradient between extra- and intravascular space, ASN levels are subsequently reduced in the extravascular spaces, e.g. the cerebrospinal fluid [Schwartz, 1970].

Several studies concerning the distribution of ASNase preparations in different species have been described in literature [Broome, 1968]; [Pütter, 1970]; [Pütter J, 1970]. The metabolic disposition and elimination of ASNase are unknown. The disappearance of ASNase activity from blood is at least partly due to the distribution of the enzyme into the extravascular fluid and clearance via the reticuloendothelial system. No accumulation in the body and no excretion in the urine were observed in ASNase-treated

dogs. The drug is partly metabolised by plasma proteases as it was first demonstrated with purified kathepsin isolated from beef spleen extract [Pütter, 1970]. More recently, two lysosomal cysteine proteases, cathepsin B (CTSB) and asparaginyl endopeptidase (AEP) have been identified in different ALL cell lines and human leukemic cell lysates. Both proteases degrade ASNase. This pathway of degradation may also be responsible for the generation of the antigenic protein fragments for anti-asparaginase antibody production [Patel, 2009].

## 2.3.4. Toxicology

Toxicity studies have been conducted with rASNase preparations in rat and dog following intravenous and intramuscular injection to reflect the intended route for human use. Additionally, subcutaneous, paravenous and intra-arterial administration have been applied to assess potential toxicity at injection sites made accidentally.

***Single dose toxicity***

A single dose toxicity study in rats was performed to evaluate acute overdosing in humans.

**Table 4: Summary of findings from single dose toxicity study LPT 16366/02**

| Study ID | Species/ Sex/Number/ Group | Dose (U/kg) /Route (Batch used) | Approx. lethal dose / observed max non-lethal dose | Major findings |
|---|---|---|---|---|
| LPT 16366/02 (GLP) | CD rats rats/5/sex/group | 30,000<br>60,000<br>120,00o<br>i.v. bolus<br>(rASNase MC1102) | >120,000 U/kg | A single i.v. injection of MC1102 revealed no toxic symptoms and no mortality up to a dose level of 120,000 U/kg b.w.<br>All animals gained the expected body weight.<br>No macroscopic changes were noted at necropsy. |

Data from the literature showed the effects of asparaginase in several species(see table below). Asparaginase was associated with several toxicities, including hyperglycaemia, hypolipoproteinaemia, hypoalbuminaemia, coagulation factor deficiencies, hepatotoxicity and pancreatitis, respectively. The LD50 values of acute toxicity studies with ASNase in different species are summarised in the table below.

**Table 5: Acute toxicity of L-asparaginase (LD50) derived from the literature**

| Species | Intraperitoneal [IU/kg] | Intravenous [IU/kg] | subcutaneous [IU/kg] | Reference |
|---|---|---|---|---|
| Mouse | 2,000,000 | 850,000 | 2,000,000 | Ohguro Y et al., 1969 |
| Mouse | - | > 200,000 | - | Lorke Det al., 1970 |
| Mouse | - | 500,000 | - | Merck, 2000 |
| Rat | 400,000 | 380,000 | 450,000 | Ohguro Y et al., 1969 |
| Guinea pig | 90 | > 150,000 | - | Ohguro Y et al., 1969 |
| Rabbit | - | 1,000 | - | Ohguro Y et al., 1969 |
| Rabbit | - | 500 - 10,000 | - | Lorke Det al., 1970 |
| Rabbit | - | 22,000 | - | Merck, 2000 |
| Cat | - | 50,000 | - | Lorke Det al., 1970 |
| Dog | - | 50,000 | - | Lorke Det al., 1970 |

All species tested generally exhibited varying degrees of decreased activity, weakness, anorexia, and weight loss. Mice and rats were found to tolerate extremely large doses of ASNase without any apparent damage to tissues. Acute toxic effects consisted of limb spread, flaccidity, and hyperpnea [Schein et al., 1969]. In cats and dogs, decreased motility, anorexia, weakness and weight loss was observed as well as vomiting. Doses of up to 10,000 IU/kg were tolerated without significant adverse effect other than vomiting [Lorke, 1970].

Rabbits appeared very sensitive to ASNase administration. The $LD_{50}$ value was substantially lower than that observed in the mouse. In a pyrogen assay of 14 different enzyme batches, a syndrome consisting of papillary constriction, profuse salivation, convulsions, respiratory paralysis, and death, occurred over a period of 24 – 72 hours after drug administration and was observed in 11 animals (22 %) receiving either 167 or 200 IU/kg i.v. The remaining 40 animals were observed for 7 days after injection, during which they showed no overt signs of toxicity [Oettgen, 1967].

Fifty percent of New Zealand white rabbits died between 24–47 hours after a single intravenous injection of 200 IU/kg and 1,000 IU/kg of ASNase [Oettgen, 1967; Chisari, 1972; Young, 1973]. The parathyroid was identified as the organ targeted by the enzyme. Similar damage of the parathyroid gland (hypoparathyroidism) was manifested in 40 Chinchilla rabbits after administration of 1,000 or 5,000 IU/kg ASNase by way of tetanic symptoms, hypocalcaemia, hypomagnesaemia, and in most cases by hyperphosphataemia followed by increased mortality [Tettenborn, 1970].

***Repeat dose toxicity***

Repeat-dose toxicity studies were provided and corresponding toxicokinetic evaluations have been performed.

An overview of studies 16367, 16368 and 16374 is presented below.

**Table 6: Summary of findings following repeated administration of asparaginase for 4 weeks**

| **Study ID** | **Species/ Sex/Number/ Group** | **Dose (U/kg/day) /Route (Batch used))** | **Approx. lethal dose / observed max non-lethal dose** | **Major findings** |
|---|---|---|---|---|
| LPT 16367/1/02 (4 weeks) (GLP) | CD rat<br>10/sex/group main<br>5/sex/group recovery (control high dose only)<br>4 weeks period<br>3/sex/group TK | 100, 1000, 10,000<br>i.v. bolus<br>**(rASNase MC1102)** | NOEL < 100 | No mortality<br>100 U/kg: clinical signs: skin lesion in the neck and nape area; haematology: influence in both sexes (number of leucocytes, fibrinogen content), thromboplastin time (M), number of reticulocytes (F); influence on biochemistry parameters (M: total protein, F: creatinine levels); ↓ spleen weight (both sexes)<br>1000 U/kg: ↓ body weights (F), ↑ food consumption (F), ↑ cholesterol levels (both sexes), ↓ alkaline Phosphatase (M)↑ relative brain weight (F)<br>10,000 U/kg: additional signs of toxicity (pilo-erection and rough fur);↓ body weight (both sexes); ↑ food consumption (both sexes); biochemistry: additional changes activity of ALAT (both sexes); ↑ urinary pH value (M) |

| LPT 16368/1/02 (4 weekls) (GLP) | Beagle dog rats/5/sex/group | 50, 150, 450 i.v bolus **(rASNase MC1102)** | NOEL>450 | No mortality, no influence on body weight, food and drinking water consumption, heart rate, circulatory functions, haematological and biochemical parameters, eyes or optic region, urinary status and volume, relative or absolute organ weights; no pathological findings at macroscopy and histopathology. |
|---|---|---|---|---|
| LPT 16374/1/02 | CD rat 10/sex/group main 5/sex/group recovery (control high dose only) 4 weeks period 3/sex/group TK | 100, 1000, 10,000 i.v. bolus (ASNase medac) | NOEL < 100 | 100 U/kg: clinical signs: pilo-erection and skin lesion in the neck and nape area; haematology: influence in both sexes (number of leucocytes, fibrinogen content), thromboplastin time (M), ratio of reticulocytes, haemoglobin content, number of erythrocytes, haematocrit value (F); influence on biochemistry parameters (M: Na and total protein, F: Ca levels); ↓ spleen weight (both sexes), i↑ thymus and kidney weights (F) 1000 U/kg: ↓ body weights, ↑ food consumption (F), ↑ cholesterol levels (both sexes), ↓ spec. gravity and ↑ urine volume (F) 10,000 U/kg: severe signs of toxicity (↓ motility and sedation, including apathy and lateral position, pilo-erection, rough fur, skin lesions); ↓ body weight (both sexes); biochemistry: additional changes in potassium (F) and alkaline Phosphatase (M); ↓ spec. gravity and ↑ urine volume (F); in addition, ↑ relative brain weight (F) |

The antigenicity of rASNase was determined in the course of four-week toxicity studies (in rats and dogs). The determination of anti-asparaginase antibody titers (non-GLP) in rats revealed an increase of anti-asparaginase antibody titers only in low-dose treated animals (100 U MC1102/kg/day) on test Days 21 and 28. Intermediate and high-dose treated rats (at ≥ 100 U/kg) did not show an increase of antibody titers at any time and it was suggested that the detection of anti-dug antibodies may not be possible in the groups of animals given the maximum dose of 10000 U/kg/day. In dog, the observed ADA induction was independent of dose and was evident up until the last day of dosing (Day 28).

Adverse effects on the liver (e.g. morphological evidence of cytolysis), alterations of the endoplasmic reticulum in spleen and thymus, and a clear increase in the content of cellular lipid were observed in Wistar rats following intraperitoneal administration of the enzyme, but rats did not show evidence of significant immunogenic toxicity [Celle et al., 1973; Cooney DA et al., 1975].

L-asparaginase caused effects on the parathyroid in a large percentage of rabbits. Primary findings associated with ASNase treatment included body weight loss, decreased food consumption, nausea, diarrhoea and hypersensitivity at the higher doses. On pathohistological examination, fatty disposition in liver cell and change of reticulum cells in spleen and thymus were observed without dose response relation [Ohguro, 1969]. In a separate publication, intravenous administration of E. coli ASNase to rabbits resulted in high incidence of hypocalcaemia (100 %), and tetany (70 %) [Young, 1973].

Anaphylactic episodes have been reported [Cooney, 1975]. Rhesus monkeys treated intravenously with ASNase developed varying degrees of weight loss, colitis, leukopenia, and abnormal liver function. Anorexia and weight loss were associated with therapy, and were generally reversible [Schein, 1969].

***Genotoxicity***

No genotoxicity studies were provided but literature data have been presented.

The mutagenicity of ASNase was tested in the Ames microbial mutagenicity test using *Salmonella typhimurium* strains TA98, TA100, and TA92. L-asparaginase (152 IU, 455 IU, 909 IU per plate) was not mutagenic to either strain with or without S9 mix used for metabolic activation. Moreover, it had no lethal effect at doses of up to 3 mg/plate (909 IU) [Seino, 1978]. Similar results were observed when ASNase was evaluated at 5, 50 and 500 µg (1.51, 15.1, 151 IU) per plate using *Salmonella typhimurium* strains TA98 and TA100 [Pak, 1979].

In a separate study, the chromosomal analysis of 7 pregnant New Zealand white rabbit females and fetuses treated with ASNase (50 or 100 IU/kg i.v.) showed normal karyotypes. *In vitro*, ASNase decreased the mitotic index of rabbit white blood cell cultures without altering the karyotype. Furthermore, analysis of liver preparations from 20 fetuses obtained from the treated does revealed normal karyotypes even though 3 fetuses had gross malformations [Adamson, 1970].

***Carcinogenicity***

No carcinogenicity studies were provided.

***Reproduction Toxicity***

The embryotoxic and teratogenic effect of native *E. coli* ASNase was well established since the 1970s and was studied *in vitro* as well *in vivo* at different stages of pregnancy in mice, rats, chicken and rabbits [Adamson, 1968a; Adamson, 1970; Ohguro., 1969; Lorke, 1970; Sanfeliu, 1986, 1989; Domenech Mateu, 1974].

Embryotoxicity studies which were conducted mainly with ASNase preparations from *E. coli* suggest a potential for teratogenicity in different species. The spectrum of results in mammalian species ranges from "no finding" in the rat [Adamson, 1968a; Adamson, 1970] to the appearance of malformations, mainly in the central nervous system (spina bifida, hydrocephalus) as well as cardiac and skeleton anomalies, gastroschisis, and missing tail among the offspring of mice, rats, rabbits and chicken, treated with L-asparaginase during pregnancy [Adamson, 1970; Ohguro, 1969; Sanfeliu, 1986; Sanfeliu, 1989]. [Domenech Mateu, 1974].

***Toxicokinetic data***

*Toxicokinetics and Interspecies Comparisons*

The Applicant presented a table which compares exposures observed in the dog to those observed in man.

**Table 7: Comparison of $C_{max}$ and AUC in the dog *vs* human (adult and paediatric patient)**

| | Factor $C_{max}$ [a] | | Factor $AUC_{0-72h}$ | |
|---|---|---|---|---|
| **Species** | **Children** [b] | **Adults** [c] | **Children** [b] | **Adults** [c] |
| ***Dog*** [d]: 1000 U/kg i.v. | 7.1 | 9.1 | 9.5 | 8.1 |

[a] after 1st administration
[b] single i.v. infusion of 5,000 U/m² MC1003 in ASNase-naive paediatric patients with haematological neoplasia (MC-ASP.4/ALL)
[c] single i.v. infusion of 5,000 U/m² MC1003 in ASNase-naive adult patients with haematological neoplasia (MC-ASP.2/RHN)
[d] data from pharmacokinetic study with rASNase MC1003 (see section 2.4.3.2)
Factor AUC = $AUD_{0-72h}$ (dog) / $AUC_{0-\infty}$ (human)
Factor $C_{max}$ = $C_{max}$ (dog) / $C_{max}$ (human)

### *Local Tolerance*

The Applicant provided a series of local tolerance studies which are outlined below.

**Table 8: Local Tolerance studies**

| Type of Study | Study No. | Species | Route of Admin | Dose | GLP-Status | Batch No. rASNase |
|---|---|---|---|---|---|---|
| *Local Tolerance* | | | | | | |
| Local tolerance test (incl. i.m. route) of MC0703 in rats (2004) | LPT 17237/03 | Rat | i.v., i.m., s.c., i.a., p.v. | Single | Yes | MC0703 |
| Local tolerance test (incl. i.m. route) of Asparaginase 10,000 medac® in rats (2006) | LPT 17697/04 | Rat | i.v., i.m., s.c., i.a., p.v. | Single | Yes | Asparaginase 10000 E medac |
| Local tolerance test of MC1005 in rats (2004) incl. amendment No. 01 (2007) | LPT 20153/06 | Rat | i.v., i.m., s.c., i.a., p.v. | Single | Yes | MC1005 |
| Local tolerance at injection sites during 4-week toxicity studies with Asparaginase medac, and MC1102 in rats and dogs. | | | | | | |
| Local tolerance at injection sites during pharmacokinetic studies with Asparaginase medac, MC0201and MC0703 in dogs. | | | | | | |

Single rASNase injections to various sites revealed good local tolerance in rats after i.v., i.a. and s.c. administration, respectively. The intramuscular (i.m.) and paravenous (p.v.) administration revealed a moderate inflammatory reaction.

Single Asparaginase 10000 E medac injections to various sites revealed an excellent local tolerance in the rat at the i.v. and s.c. administration sites. The i.m., i.a. and p.v. administration sites revealed a moderate inflammatory reaction. The local tolerance test of a new citrate-buffer free formulation of lyophilised rASNase MC1005 revealed an excellent tolerance following single i.v., i.m., i.a., p.v. and s.c. injection in rats. This therefore supports the intended clinical use of the final product rASNase MC1005.

No macroscopic or microscopic findings of concern were noted at the injection sites.

### *Other toxicity studies*

The potential for immunotoxicity has been described in the literature.

*Effects on antibody producing cells*

With respect to its recognized immunosuppressive properties, previous work has shown that in mice, ASNase preferentially inhibits antibody-precursor cells in the bone marrow (Friedman, 1971), and inhibits both cell-mediated and humoral immune responses. A considerable decrease of antibody-producing cells (about 90 %) was observed in a 3-month toxicity study in male and female Wistar rats. Animals were treated daily for 7 days with 3,200 IU/kg (i.p.) ASNase from *E. coli* or from *Erwinia carotovora*. The effect was roughly dose-dependent and apparently more marked after treatment with L-ASNase from *E. coli*. The immunosuppressive effect of ASNase appeared to diminish with treatment, especially in rats treated with the *Erwinia carotovora* enzyme, and the lower dose was more immunogenic than the higher one for both types of enzymes (Cavanna, 1976).

*Effects on T or B lymphocyte response to mitogen stimulation*

L-asparaginase has long been known to be a potent inhibitor of the *in vitro* response of lymphocytes to mitogen stimulation. The mechanism of inhibition of PHA- or SAC-stimulated lymphocyte blastogenesis by ASNase has been reported not to be ASN depletion but GLN depletion [Kitoh *et al*., 1992].

*Immunosuppressive effects of asparaginases*

Several studies have shown immunosuppressive effects of ASNase on both humoral and cell-mediated immune reactions [Durden, 1980, 1981].

### 2.3.5. Ecotoxicity/environmental risk assessment

In accordance with the Guideline on Environmental Risk Assessment (ERA) of Medicinal Products for Human use [CHMP/SWP/4447/00] the Applicant submitted a justification for not submitting an ERA. Considering asparaginase is a recombinant protein, it is unlikely to result in a significant risk to the environment. The justification was considered acceptable.

### 2.3.6. Discussion on non-clinical aspects

Based on the data presented, the enzymatic and structural properties of the proposed product, recombinant asparaginase appear to be similar to that of the asparaginase preparation currently on the market derived from Escherichia coli (asparaginase 10,000E medac). Overall, the batches of recombinant asparaginase showed similar anti-proliferative profiles, when compared to the asparaginase medac, whereby they both caused a dose-dependent inhibition of cell proliferation at 0.001 to 100 U/mL. However, the anti-proliferative activity afforded by one of the batches (MC0703) at 0.01 and 100 U/mL was slightly lower than that of asparaginase medac. However, observed difference between the proposed product and the asparaginase medac are not clinically relevant. Follow up investigative in vivo studies suggested that in a single species only (the mouse), it is the high molecular weight aggregates within asparaginase medac (which typically contains ~20% aggregates) that were responsible for the increased efficacy (when compared to that of the proposed product) (see discussion below on pharmacokinetic aspects).

No secondary pharmacodynamic studies were submitted. Since chemical structure and *in vitro* activity of recombinant ASNase is very similar to that of the ASNase Medac, an extensive evaluation of effects at unintended molecular targets is not deemed necessary.

Non-clinical repeat-dose toxicity and safety pharmacology studies in rats revealed no special hazard for humans, except a slight but significant saluretic effect at doses below the recommended dose for ALL/LBL patients. Additionally, the urinary pH value and the relative weight of kidneys were increased at exposures considered sufficiently in excess of the maximum human exposure indicating little relevance to clinical use (see SmPC section 5.3).

Additional safety pharmacology data from the literature showed that asparaginase has the potential to cause hyperglycaemia (and hypoinsulinemia), acute hypersensitivity reactions (and possibly pancreatitis) and disturbances in hepatic function (e.g. increased liver lipid levels and decreased plasma levels of albumin) and clotting (subsequent to a deficiency of antithrombin III). With the exception of the effects on insulin levels, the observed effects occurred at doses that were greater than those proposed clinically (on a $U/m^2$ basis). These observed effects are included in the SmPC (see SmPC section 4.8).

No new pharmacodynamic drug interaction studies were performed with r-asparaginase. However, the potential for pharmacodynamic interactions with other chemotherapeutic agents has been described based data from the literature. Inhibition of protein synthesis secondary to the asparaginase-induced depletion of asparagine has been shown to attenuate the cytotoxic effect of MTX which requires cell replication for its antineoplastic activity. This antagonism is observed if asparaginase is administered prior to or concurrently with methotrexate. Conversely, the antitumour effects of methotrexate are enhanced when asparaginase is administered 24 hours following methotrexate treatment. This regimen has been shown to reduce the gastrointestinal and haematological effects of methotrexate. (see SmPC section 4.5).

In addition, there is *in vitro* and *in vivo* evidence to suggest that the therapeutic/anti-leukaemic effects afforded by the anti-metabolic agent, cytosine arabinoside (ara-C) and asparaginase are schedule-dependent. Laboratory *in vitro* and *in vivo* data indicate that the efficacy of high-dose

cytarabine is reduced by prior administration of asparaginase. However, when asparaginase was given after cytarabine a synergistic effect was observed. This effect was most prominent with a treatment interval of about 120 hours. (see SmPC section 4.5).

In the mouse, the frequency of osteonecrosis after 6 weeks of dexamethasone plus asparaginase was comparable to that observed following 12 weeks of dexamethasone alone, which suggests that concomitant administration of dexamethasone and L -asparaginase in mice accelerated the potential onset of glucocorticoid-induced osteonecrosis. A synergistic anti-leukaemia effect has been shown *in vitro* when vincristine is administered with asparaginase. In addition, synergism has been observed *in vitro* and *in vivo* when vincristine, dexamethasone and L-asparaginase are administered in combination. The toxicity of vincristine may be additive with that of asparaginase if both agents are administered concomitantly. Therefore, vincristine should be given 3 to 24 hours before administration of asparaginase in order to minimise toxicity. (see SmPC section 4.5).

Regarding pharmacokinetic studies, the analytical methods used appear to have been adequately validated and are considered suitable for use in the determination of L-asparaginase activity. Potential issues were noted with respect to the ELISA used to determined anti-drug antibody levels and it was subsequently clarified that the antibody profiling was of a preliminary nature as the antibody determinations were not performed to GLP. To aid with the characterisation of the pharmacokinetic profile, serum levels of asparagine, aspartic acid, glutamine and/or glutamic acid were also determined using reverse phase high pressure liquid chromatography (RP-HPLC). In the NOD/SCID mouse, the observed anti-leukaemic efficacy correlated with a high content of aggregates in the asparaginase formulations which displayed significantly higher retention times or half-lives, lower clearance and higher AUC values (than that observed in low-aggregate preparations). It is noted no non-clinical pharmacokinetic evaluation was performed on the rASNase batch intended to be commercialised. However, in light of the fact that comparable results have been adequately demonstrated from a quality perspective, no further non-clinical studies are warranted.

Overall, following repeated administration, the toxicokinetic data generated suggest that there is no indication of persistent accumulation of recombinant asparaginase in the rat and dog; there was in fact, a tendency towards a decrease in serum activity over time.

Based upon the available data on asparaginase and the fact that the proposed product is expected to possess similar pharmacokinetic properties to that of currently marketed forms of asparaginase, the Applicant did not perform any additional distribution, metabolism, excretion or pharmacokinetic drug interaction studies. This approach is deemed to be acceptable.

The bigger the molecule, the greater the chance of a hypersensitivity reaction. Given that the risk of hypersensitivity is likely to be of most concern, Section 4.8 of the SmPC and the Risk Management Plan inform of this risk.

Due to the bacterial origin of asparaginase preparations, an antigenic potential resulting in the induction of a humoral immune response was expected. Although discrepancies in the reporting of these data were noted and subsequently clarified, overall in the rat, a significant increase in anti-asparaginase antibody titre was induced only at the lowest dose of 100 U/kg, whereas a strong increase in anti-asparaginase antibody titre was observed in all 3 dose groups in the dog (50 to 450 U/kg). It is speculated that serum antibodies in rats may be absorbed completely, when excessive levels of antigen (asparaginase protein) are present in the serum and corresponding toxicokinetic data supported this hypothesis. In contrast, in the dog, comparably low doses of asparaginase (maximum dose of 450 U/kg) may not have been sufficient to absorb the anti-asparaginase antibodies.

The evidence from the literature suggests that asparaginase and therefore the proposed product should not cause genotoxicity. The Applicant has not conducted additional studies, which is in accordance with

the recommendations of ICH S6. In addition, in light of the therapeutic indication claimed, the post-marketing experience gained with native asparaginase and its protein nature, the lack of carcinogenicity study is acceptable. Overall, evidence from published data with asparaginase renders the mutagenic, clastogenic and carcinogenic potential of asparaginase negligible (see SmPC section 5.3).

Following repeated administration in the rat and dog, histological examination of the testis and the ovary did not reveal any pathological findings; however, given that the maximum dose administered to the dog is below that proposed clinically (on a $mg/m^2$ basis), and given the pharmacological activity of asparaginase, an effect on fertility cannot be completely ruled out. Hence, Section 4.6 of the SmPC advises both males and females to use effective means of contraception.

No investigation was performed on the influence of fertility and on the potential transfer of this agent to breast milk; the proposed product should not used during lactation in humans. There is extensive information available regarding the potential effects of *E. coli* asparaginase on embryofetal development. The potential for growth retardation, an increased risk of malformations (including those of the central nervous system, heart and skeletal system) and foetal death is evident from the *ex vivo* and *in vivo* studies performed at doses that are similar to or in excess of those proposed clinically (on a $U/m^2$ basis) in a number of species including the mouse, rat and/or rabbit. The potential for a risk to embryofetal development is reflected in Section 5.3 of the SmPC. Section 4.6 of the SmPC advises that patients shall use contraceptive measures during and up to 3 months after cessation of rASNase containing chemotherapy and also warns against the use of the proposed product during pregnancy; overall, this approach is acceptable. It is agreed that based on the information available in the public domain that no further non-clinical data to describe the effects on embryofetal development are warranted.

A series of studies have been conducted in order to assess the local tolerance following intravenous, paravenous, intramuscular, intra-arterial and subcutaneous administration of recombinant asparaginase. No macroscopic or microscopic findings of concern were noted at the injection sites.

Asparaginase preferentially inhibits antibody-precursor cells in the bone marrow and inhibits both cell-mediated and humoral immune responses. Glutamine hydrolysing activity of E. coli asparaginase may contribute significantly towards this immunosuppression. Beside the depletion of ASN, *E. coli* asparaginase reduced circulating glutamine concentrations and reduced protein synthesis in liver and spleen but not in pancreas assuming that the nutrient stress response to asparaginase is tissue-specific and exacerbated by glutamine depletion. The SmPC notes the potential for infections which addresses the potential for immunosuppression; hence from a non-clinical perspective, no further action is required.

### 2.3.7. Conclusion on the non-clinical aspects

Overall, the non-clinical package submitted to support this application is acceptable.

## *2.4. Clinical aspects*

### 2.4.1. Introduction

***GCP***

The applicant claimed that the clinical trials were performed in accordance with GCP. A GCP inspection was triggered on the pivotal study MC-ASP.5/ALL and MC-ASP.6/INF. The inspection uncovered several findings. One critical finding occurred during inspection of the bioanalytical laboratory and concerned non-adherence to the study plan criteria for accepting or rejecting calibration standards for the

asparaginase assay. Due to these findings the pivotal study MC-ASP.5/ALL was not considered to be GCP compliant (see discussion on clinical efficacy).

- Tabular overview of clinical studies

| Study ID | No. of study centres/ locations | Design | Study Posology and formulation used | Study Objective | N.of subjects | Treatment duration | Gender M/F Median Age | Diagnosis Incl. criteria | Primary Endpoint |
|---|---|---|---|---|---|---|---|---|---|
| **MC-ASP.5/ALL** | 7 sites<br><br>Netherlands | Double-blind, controlled, phase III | rASNase (**MC0707**) vs Asparaginase medac<br><br>**5,000 U/m²** q3d x8 (Days 12, 15, 18, 21, 24, 27, 30 and 33)<br><br>**Post-induction**:<br>**SR**: pegaspargase day 1<br>**MR**: pegaspargase 15 infusions<br>**HR**: rASNase or ASNase medac **10,000 U/m²** (4 infusions in blocks 1, 2, 4, 5 and protocol II)<br><br>IV | Rate of complete ASN depletion (non-inferiority) | 199 (total)<br><br>98 (rASNase)<br><br>101 (Asparaginase medac) | 22 days (induction)<br><br>In HR pts: 11 days in each block | 107/92<br><br>rASNase: 54/44<br>5 (1-17)years<br><br>ASN medac: 53/48<br>4 (1-16) years | Children (≥1≤18 years)<br><br>de novo ALL<br><br>previously untreated | Rate of patients with complete ASN depletion in serum during induction treatment |
| **MC-ASP.4/ALL** | 1 site<br><br>Netherlands | Double-blind, controlled, phase II | rASNase (MC1003) vs Asparaginase medac<br><br>**5,000 U/m²** q3d x8 (Days 12, 15, 18, 21, 24, 27, 30 and 33) | PK/PD, efficacy, safety | 32 (total)<br><br>16 (rASNase)<br><br>16 (Asparaginase medac) | 22 days | 17/15<br><br>4.5 years (rASNase)<br><br>4.5 years (Asparaginase medac) | Children (≥1≤18 years)<br><br>de novo ALL | AUC $_{0\text{-}72h}$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | IV | | | | | | |
| **MC-ASP.6/INF** | 17 sites<br><br>Netherlands (6)<br>Germany (11) | Non-controlled, phase II | rASNase (MC1003)<br><br>**10,000 U/m²**<br>q3d x 6<br>(days 15, 18, 22, 25, 29, 33)<br><br>IV | efficacy, safety | 12 | 19 days | 7/5<br><br>6 months (0.5-12.2 months) | Infants (<1year)<br><br>de novo ALL<br><br>previously untreated | Number of pts with hypersensitivity reactions to rASNase during induction treatment |
| **MC-ASP.1/ALL** | 5 sites<br><br>Germany<br><br>(patients enrolled in only 1 site) | Open-label, non-controlled, phase II | rASNase (MC1003)<br><br>**10,000 U/m²**<br>Days 7, 8, 14, 15<br><br>IV | PK/PD, efficacy, safety | 2 | 9 days | 1/1<br><br>22 years<br>59 years | Adults patients<br>ALL late relapse | Number of patients with complete ASN depletion in serum during induction treatment |
| **MC-ASP.2/RHN** | 1 site<br><br>Germany | Non-controlled, phase I/II | rASNase (MC1003)<br><br>**5,000 U/m²** q3d x maximum 8 infusions<br><br>IV | PK/PD, efficacy, safety | 7<br><br>(NHL:5; AML:2) | 22 days (maximum) | 5/2<br><br>72 years (56-77) | Adults patients<br>Relapsed/refractory/indolent haematologic neoplasias | $AUC_{0-72h}$ |

### 2.4.2. Pharmacokinetics

***Absorption***

Asparaginase is not absorbed by the gastrointestinal tract. Bioavailability of recombinant asparaginase after IM administration has not been studied.

***Distribution***

In study MC-ASP.4/ALL, in 14 children/adolescents (age 2-14 years) with *de novo* ALL, the median volume of distribution of r-L-asparaginase at steady state ($V_{dss}$) was 0.95 l (range: 0.7 – 2.8 l).

The mean (SD) of $V_{dss}$ in seven adult patients in study MC-ASP.2/RHN was 2.47 l (0.45 l) after administration of 5,000 U/m² r-L-asparaginase.

In these two studies, after administration of 5,000 U/m² r-L-asparaginase, median (range) maximum serum concentrations of asparaginase activity were 2,324 U/l (1,625 – 4,819 U/l) in 7 adult patients and 3,350 U/l (2,231 – 4,526 U/l) in 14 children/adolescents respectively.

In adult patients, the peak ($C_{max}$) of asparaginase activity in serum was reached with a delay of approximately 2 hours after the end of the infusion whereas in most children/adolescents $C_{max}$ was reached at the end of infusion.

A summary of the PK parameters reported in study ASP.2/RHN and study ASP.4/ALL is presented below.

*PK parameters of rASNase in adults versus children (median [range])*

| Study | ASP.2/RHN | ASP.4/ALL |
|---|---|---|
| *Data source* | CSR table 11.4.1-A | CSR table 14.2.1.2 |
| Number of patients | 7 adults | 14 children |
| $AUC_{0-72h}$ [U*h/L] | 57,306 [45,512 - 78,275] | 60,165 [38,627 - 80,764] |
| $C_{max}$ [U/L] | 2,324 [1,625 – 4,819] | 3,527 [2,231 - 4,526] |
| $T_{max}$ [h] | 2.33 [0.43 – 6.75] | 0 [0 – 2] |
| $T_{1/2\ \lambda z}$ [h] | 26.0 [14.2 – 44.2] | 17.33 [12.54 - 22.91] |
| $Cl_{tot}$ [L/h] | 0.073 [0.056 – 0.106] | 0.053 [0.043 – 0.178] |
| $V_{dss}$ [L] | 2.635 [1.878 – 3.000] | 0.948 [0.691 – 2.770] |

After repeated administration of asparaginase at a dose of 5,000 U/m² every third day, trough asparaginase activity levels in serum ranged from 108 to 510 U/l in adult patients.

In trial MC-ASP.5/ALL, samples of CSF were taken at day 33 of induction treatment - the day of the last administration of r-asparaginase or Asparaginase medac. Asparaginase activities in CSF on Day 33 were below the lower limit of quantification in nearly all patients. Only three of 176 evaluable samples (two in the reference group and one in the r-asparaginase group) contained measurable amounts of asparaginase activity on Day 33 of induction treatment.

***Elimination***

Metabolism

Patel et al. reported that two lysosomal cysteine proteases present in lymphoblasts are able to degrade asparaginase. Cathepsin B, which is produced constitutively by normal and leukaemic cells, degraded asparaginase produced by Escherichia coli and Erwinia chrysanthemi. Asparaginyl endopeptidase (AEP) specifically degraded E. coli-derived asparaginase. The N24 residue on the flexible active loop was identified as the primary AEP cleavage site. Sole modification at this site rendered asparaginase resistant

to AEP cleavage and suggested a key role for the flexible active loop in determining asparaginase activity. AEP is very variably expressed in children with ALL, with highest activity observed in high risk patients (Patel et al. 2009).

In 7 adult patients in study MC-ASP.2/RHN, the mean ± SD terminal half-life (elimination half-life) of asparaginase activity in serum was 25.8 ± 9.9 h, with a range between 14.2 and 44.2 h.

In 14 children/adolescents in study MC-ASP.4/ALL, the mean ± SD terminal half-life (elimination half-life) of asparaginase activity in serum was 17.1 ± 1.2 h, with a range between 12.5 and 22.9 h.

Excretion

Asparaginase is thought to be degraded within the reticulo-histiocytic system and/or by endopeptidases present in the serum (Patel et al. 2009). Only minimal urinary and biliary excretion of asparaginase was found in one published investigation in dogs (Ho et al. 1971). In the studies performed, recombinant asparaginase levels were not measured in urine or faeces of patients.

***Dose proportionality and time dependencies***

Data are available from a published clinical trial with the reference drug Asparaginase medac. Children with de novo ALL were treated with 8 doses of 10,000 U/m² (n = 39 patients), 5,000 U/m² (n = 15 patients), or 2,500 U/m² (n = 11 patients) Asparaginase medac during induction treatment. Mean ± SD trough asparaginase activity levels were 430 ± 300 U/L, 270 ± 109 U/L, and 125 ± 68 U/L (Ahlke et al. 1997).

**Intra- and inter-individual variability**

A population pharmacokinetic (PopPK) two-compartment model for recombinant asparaginase was developed based on asparaginase-activity levels in serum obtained in the three clinical trials (MC-ASP.4/ALL, MC-ASP.5/ALL, MC-ASP.6/INF) with recombinant asparaginase in children with de novo ALL. These children received recombinant asparaginase at a median dose of 5,000 U/m² (range 4,615 – 10,000) during induction treatment. Data of the 3 trials were merged in a NONMEM-dataset. The dataset included the actual infusion- and sampling-times of 640 serum samples from 124 children.

The final structural-model was a two-compartment model for recombinant asparaginase (rASNase). Even though two physiological distribution areas appeared improbable for rASNase, the superiority of the two-compartment-model indicated that two mechanisms with different speeds may be involved in the rASNase elimination. In most patients an initial distribution phase with a short elimination half-life of about 0.2 hours was apparent. Shortly thereafter, the rASNase-elimination followed simple first-order kinetics with a terminal elimination half-life of 17.4 hours.

The base model was a one-compartment model with interindividual variability estimated on clearance (CL) and central volume of distribution (V1) and a combined error model (additive and proportional). The final base structural PK model was a 2-compartment model with IIV estimated on CL and V1 and interoccasion variability (IOV) on CL. Body weight was included as a scaling factor for all structural parameters (CL, V1, Q and V2).

The parameter estimates for the final population PK model for rASNase-activity were provided. The relative standard errors for all estimated PK-parameters were smaller than 22%.

The plots of the observed and model-predicted rASNase-activities of the individual patients from the trial with more intensive sampling (trial 1 (study number MC-ASP.4/ALL) with 16 children between 2 and 14 years) and trial 3 (study number MCASP. 6/INF) (with sparse sampling in 12 infants between 3 days and 1 year)) showed that the model can adequately estimate the observed rASNase trough activities.

Parameter estimates were: CL 0.202 l/h/70 kg, V1 0.933 l/70 kg, Q 2.87 l/h/70 kg and V2 3.91 l/70 kg. The inter-individual and inter-occasion variability of the CL were relatively low with 14.8 % and 6.88 %, respectively. In contrast, inter-individual variability of V1 was high (66.6 %), mainly reflecting the variability in the peak plasma values ($C_{max}$).

Residual variability was calculated with a combined error model (additive error 13.5 U/l; proportional error 6.07 %). Body weight (WT) was used as scaling factor on the PK-parameters CL and Q (factor 0.0378) as well as on the V1 and the V2 (factor 0.0450). The comparison of a model with WT as scaling factor with a model that included the scaling factor body surface area (BSA) did not show a marked model-deterioration. The objective function only increased by one point and IIV of the V1 as well as the relative standard errors of the estimated values for the CL, the Q, the V2 and the residual error were slightly larger in the model including the BSA than in the model using the WT as scaling factor.

<u>Influence of demographic covariates on the asparaginase-activity</u>: The model-predicted individual trough values showed no correlation with the covariates age, body surface area (BSA), body weight and body height (model-predicted individual values more than 43 hours after recombinant asparaginase-infusion). There was no significant difference between the trough levels between boys and girls. Patients receiving more than 6500 U/m² rASNase had lower trough levels than patients receiving lower doses. Patients receiving more than 6500 U/m² rASNase were all infants included in trial 3 (N=12).

<u>Influence of recombinant asparaginase trough activities on laboratory findings:</u> The measured serum creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, antithrombin III and fibrinogen values did not appear to be influenced substantially by the asparaginase-activity trough levels. Thus, from this analysis, there were no safety-concerns in this dose-schedule.

<u>Influence of recombinant asparaginase trough activities on the minimal residual disease status</u>: Patients with a negative and a positive minimal residual disease status had no significantly different asparaginase (rASNase)-activity trough levels.

<u>Influence of laboratory findings on model-predicted parameters</u>: The laboratory findings did not appear to influence the model predicted clearance (CL) and the volume of distribution of the central compartment (V1; both normalised to the body weight). However, the patients with the three highest serum creatinine values had a CL below and the patients with the three highest alanine aminotransferase concentrations had a CL above the populations´ median CL (estimated after the first asparaginase (rASNase)-infusion). The patients with the three lowest antithrombin III values had a V1 above the populations´ median V1.

<u>Infants</u>: The measured rASNase-activities of the infants included in the analysis could adequately be estimated with the final NONMEM-model. The infants receiving more than 6500 U/m² rASNase (infants included in trial 3), had lower trough activities than patients receiving lower doses. It was noted that sampling times for the trough levels were not standardised to a certain hour after administration. It should also be noted that the number of 12 infants in the dataset is small. The model predicted a significantly ($p < 0.05$) higher CL and a lower V1 for the infants.

<u>Comparison with native Escherichia coli asparaginase:</u> A comparison of the estimated pharmacokinetic (PK)-parameters of recombinant asparaginase (rASNase) with the previously published results of an analysis for native E. coli asparaginase, showed similar values for the clearance and the terminal elimination half-life (17.2 vs. 19.0 h). Lower values for V1 and higher levels for V2 were observed for rASNase in comparison to the E. coli asparaginase. However, the shape of the activity vs. time curve appeared to be similar to the E. coli asparaginase.

The large IIV of the V1 and the graphs and the plots, reflected the high variability in the peak plasma concentrations. However, the lower IIV in the CL determined a lower variability in the clinically more relevant trough concentrations and the time above the threshold activity of 100 U/l (Riccardi et al.

showed in five rhesus monkeys that an activity of 100 U/l was necessary to reach a complete asparaginase-depletion in serum and liquor).

***Special populations***

The pharmacokinetics of asparaginase was studied in 14 children/adolescents (age 2 – 14 years) with *de novo* ALL. The area under the curve of asparaginase activity in serum from 0 to 72 hours ($AUC_{0-72h}$) after first administration of 5,000 U/m² r-L-asparaginase was similar to data obtained in adult patients. Median (range) of $AUC_{0-72h}$ was 58,357 (38,627 – 80,764) U per h/l in children versus 59,632 (45,512 – 78,275) U per h/l in adults.

Terminal half-life of asparaginase activity in serum was slightly shorter in children than in adults (mean ± SD: 17.1 ± 1.2 h vs. 25.8 ± 9.9 h). Peak serum asparaginase activity was reached immediately after end of infusion in most paediatric patients.

Median trough serum asparaginase activities were measured in 81 children/adolescents with de novo ALL three days after infusion of asparaginase (just before the next dose had to be given) during induction treatment and ranged from 168 to 184 U/l (study MC-ASP.5/ALL).

In study MC-ASP.6/INF, trough serum activity levels were measured in 12 infants (age from birth to 1 year) with *de novo* ALL. Median (range) serum trough asparaginase activities on days 18, 25, and 33 were 209 (42 – 330) U/l, 130 (6 – 424) U/l, and 32 (1 – 129) U/l, respectively. The lower median activity level on day 33 compared to the former two measurements was in part due to the fact that this last serum sample was taken 4 days after the last infusion of r-L-asparaginase instead of three days on the other occasions.

***Pharmacokinetic interaction studies***

No specific pharmacokinetic drug-interaction studies with recombinant asparaginase have been submitted.

## 2.4.3. Pharmacodynamics

***Mechanism of action***

The primary pharmacodynamic effect of asparaginase is the rapid cleavage of the amino acid asparagine into aspartic acid and ammonia.

***Primary and Secondary pharmacology***

In a clinical trial in children with de novo ALL (study MC-ASP.4/ALL) it was shown that immediately after the end of infusion of asparaginase mean asparagine concentrations in serum dropped from the pre-dose concentrations of about 40 µM to below the lower limit of quantification of the bioanalytical method (< 0.5 µM). The mean asparagine concentrations in serum remained below 0.5 µM from immediately after the end of first infusion of asparaginase until at least three days after the last infusion. Thereafter, asparagine serum levels increased again and returned to normal values within 1 – 3 weeks.

In addition to asparagine, asparaginase is also able to cleave the amino acid glutamine to glutamic acid and ammonia, however with much less efficiency. Clinical trials with asparaginase have shown that glutamine levels are only moderately affected with a very high interindividual variability. Immediately after the end of infusion of asparaginase, serum levels of glutamine declined by a maximum of 50 % from pre-dose levels of about 400 µM but rapidly returned to normal values within a few hours. (see SmPC section 5.1).

*Pharmacokinetic/pharmacodynamic relationship*

In clinical trials with asparaginase, trough asparaginase serum activity levels greater than 100 U/l were achieved in the majority of patients which nearly always correlated with a complete depletion of asparagine in serum and cerebrospinal fluid (CSF). Even those few patients with trough asparaginase serum activity levels of 10 – 100 U/l usually experienced complete asparagine depletion in serum and CSF.

*Pharmacodynamic interactions*

No studies were provided. The applicant discussed available data from clinical experience with ASNase treatment.

*Corticosteroids*

Corticosteroids and ASNase are known to cause hypofibrinogenaemia. Furthermore, ASNase is known to reduce fibrinogen and antithrombin III levels. Concomitant use of corticosteroids and ASNase may increase the risk of a change in these coagulation parameters and subsequent thromboembolic events or bleeding episodes (Nowak-Göttl et al. 2009).

The use of dexamethasone instead of prednisone, administered with ASNase (Asparaginase medac), significantly reduced the onset of venous thromboembolism in trials of the German ALL-BFM study group (Nowak-Göttl et al. 2003).

A higher risk of thrombosis during induction therapy with asparaginase and prednisone was seen in children with a genetic prothrombotic risk factor (factor V G1691A-mutations, prothrombin G20210A-variation, methylenetetrahydrofolate reductase [MTHFR] T677T-genotype, increased lipoprotein A, hyperhomocysteinaemia)(see Section 4.4).

*Anticoagulants*

Concomitant use of glucocorticoids and/ or anticoagulants with asparaginase may increase the risk of a change in coagulation parameters (see section 4.4).

*Asparaginase and methotrexate*

Inhibition of protein synthesis secondary to the ASNase-induced depletion of ASN has been shown to attenuate the cytotoxic effect of MTX which requires cell replication for its antineoplastic activity. This antagonism is observed if ASNase is administered prior to or concurrently with MTX.

Conversely, the antitumour effects of MTX are enhanced when ASNase is administered 24 hours following MTX treatment. This regimen has been shown to reduce the gastrointestinal and haematologic effects of MTX (Capizzi, 1981). ASNase had no significant effect on MTX polyglutamate accumulation and MTX retention when administered after MTX (Sur et al. 1987). Clinically used combination regimens have been developed to make use of the potentially synergistic antineoplastic activity of MTX and ASNase (Harris et al. 1980; Hudson et al. 1990; Rosen et al. 2003).

*Asparaginase and cytarabine (Ara-C)*

Laboratory *in vitro* and *in vivo* data indicate that the efficacy of high-dose Ara-C is reduced by prior administration of ASNase. The mechanisms for this pharmacological antagonism may include ASNase-induced decreased cellular uptake and incorporation of Ara-C into macromolecules However, when ASNase was given after Ara-C a synergistic effect was observed. This synergism was most prominent with a treatment interval of about 120 hours, i.e. before the cells recovered from the Ara-C effect. ASNase-induced lowering of the cellular pool size of dCTP and consequent enhanced metabolism of Ara-C to its active metabolite Ara-CTP as well as down regulation of bcl-2 oncoprotein levels even in the absence of p53 are considered as possible mechanisms for this synergistic action (Schwartz et al. 1982;

Capizzi and White 1988). The therapeutic benefit of this pharmacological manipulation has been verified in several clinical trials in patients with ALL and AML (Harris et al. 1998; Wells et al. 1993).

## 2.4.4. Discussion on clinical pharmacology

**Pharmacokinetics**

As a protein, r-L-asparaginase is not absorbable from the gastrointestinal tract after oral administration. Therefore it must be given parenterally. In all clinical trials both r-L-asparaginase and asparaginase medac were administered intravenously (IV). No oral bioavailability study data are therefore available.

With regard to the possibility of administering Spectrila intramuscularly (IM), no clinical data on Spectrila bioavailability after IM administration were provided to support such use. The Applicant refered to a paper by B. Klug Albertsen (British Journal of Haematology, 2001), comparing Erwinia asparaginase and Asparaginase medac, both administered IM. The mean bioavailability after IM administration (27% for Erwinia asparaginase, and 45% for Asparaginase Medac) is significantly lower than after IV administration. To support the IM administration of Spectrila, the Applicant assumed comparable enzyme activity after the two routes of administration (IM and IV) which is not supported by the data. Furthermore, efficacy data on the use of IM asparaginase medac cannot be accepted as pivotal support for the efficacy of IM Spectrila also considering the differences in formulation and impurities. Thus, no recommendation for use of Spectrila intramuscularly can be made at this stage.

The pharmacokinetics of recombinant asparaginase were evaluated after intravenous infusion of a first dose in 14 children in study MC-ASP.4/ALL and 7 adult patients in study MC-ASP.2/RHN. Pharmacokinetic parameters in serum and CSF were calculated using non-compartmental procedures. Additionally, trough levels were evaluated in serum during induction treatment in studies MC-ASP.1/ALL, MC-ASP.5/ALL, MC-ASP.6/INF and MC-ASP.4/ALL. Pharmacokinetics results are presented and discussed under the clinical efficacy section.

The median volume of distribution of Spectrila (2.6 L in adults; 1 L in children) equals the plasma volume, suggesting that the drug is distributed mainly in the intravascular space. This likely relates to its large molecular size.

Asparaginase activity in CSF was not detected in almost all patients. This was expected as this large protein is not expected to penetrate the blood-brain barrier in measurable amounts.

In study MC-ASP.5/ALL, 3 of 176 evaluable samples (two in the reference group and one in the r-asparaginase group) contained measurable amounts of asparaginase activity in CSF on Day 33 of induction treatment. Despite that, ASN depletion in CSF was complete in more than 85% of patients. Therefore, ASN depletion by r-asparaginase appears to mainly occur within the intravascular space. Since this builds up a concentration gradient between extra- and intravascular space, ASN levels are subsequently also reduced in the extravascular spaces, e.g. the cerebrospinal fluid (Schwartz et al. 1970; Müller and Boos 1998).

The metabolism of asparaginase is not known but thought to occur via degradation within the reticulo-histiocytic system and by serum proteases. Only minimal urinary and biliary excretion of asparaginase was found in one published investigation in dogs. No new metabolism studies were therefore, performed by the Applicant and Spectrila levels were not measured in urine or faeces of patients.

Since asparaginase is degraded by plasma enzymes into its constituent amino acids, there are no active metabolites.

No dose ranging studies were submitted by the Applicant investigating PK dose linearity. However, published clinical trials suggest that asparaginase trough serum activities are linear over the dose range 2,500 to 10,000 U/m². A trough serum asparaginase activity level of greater than 100 U/L is generally considered to guarantee complete ASN depletion in serum and CSF. The majority of trough asparaginase activity levels in the combined pivotal and PK trials after repeated administration ranged from 120 – 200 U/L. PK data regarding maintenance of asparaginase level above 100 U/L have also been provided and are discussed under the clinical efficacy discussion (see also discussion on clinical efficacy).

A population pharmacokinetic (PopPK) two-compartment model for recombinant asparaginase was developed. The Applicant acknowledged the limitations of performed analyses which were mainly due to the availability of only few data from infants and to the fact that no external evaluation of the final NONMEM-model was performed due to a lack of an external dataset. The model indicated an initial distribution phase with a short elimination half-live of about 0.2 hours, and a second elimination phase by first-order kinetics with a terminal elimination half-live of 17.4 hours. Variability in the clearance estimates was relatively low, whereas inter-individual variability was significantly high (66%) for the estimates of central volume of distribution. This was somewhat expected given the known variability in the peak plasma values (Cmax).

The PopPK model showed a clear correlation between the clearance of recombinant asparaginase and body surface area (BSA), thus supporting the adequacy of dosing based on BSA. Using this PopPK model, the influence of covariates such as age, BSA, body weight, and body height on the trough asparaginase serum activities was investigated. Neither correlation with these covariates nor significant difference between the trough asparaginase serum activities of boys and girls was found. The model was also used to study the influence of trough asparaginase serum activities on status of minimal residual disease (MRD) at the end of induction treatment. Also in this case no correlation was found.

The measured serum creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, antithrombin III and fibrinogen values did not appear to be influenced substantially by the asparaginase-activity trough levels. Thus, from this analysis, there are no safety-concerns in this dose-schedule.

The small number of patients (n=12) receiving a dose of more than 6500 U/m² recombinant asparaginase had lower trough activities than patients receiving lower doses. It is notable that the patients receiving more than 6500 U/m² recombinant asparaginase were all infants (from the infant trial). This finding must be interpreted with caution, because sampling times for the trough levels were not standardised to a certain hour after administration. The model predicted a significantly ($p < 0.05$) higher CL and a lower V1 for the infants. The higher CL in infants is not surprising as the parameter was standardised to body weight and infants often show a higher clearance per kg than older children. The difference in the CL standardised to BSA was statistically significant but small and does not appear to be of clinical relevance. Therefore, from the PK point of view, there is currently no sufficient evidence to change the proposed dose in infants. The recommended dose in infants is 6,700 U/m² BSA in patients aged less than 6 months and 7,500 U/m² BSA in patients aged 6 – 12 months based on study MC-ASP.6/INF.

The Applicant did not perform any PK studies in patients with hepatic or renal insufficiency. Results from the PopPK analysis did not highlight any correlation between exposure to the two asparaginase formulations and serum creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, antithrombin III and fibrinogen covariates.

No new PK drug interaction studies have been conducted with Spectrila. No studies on induction/inhibition of drug-metabolising enzymes were performed. Well-established potential/identified interactions with medicinal products that are administered concurrently in multi-drug treatment protocols for the target indications are further discussed below and adequately reported in section 4.5 of the SmPC. This

approach is considered sufficient to justify the lack of new specific PK drug-interaction studies with asparaginase.

In view of the limited PK data in adults, the applicant will undertake further study of PK, PD and safety of Spectrila in adults post-approval (see RMP).

**Pharmacodynamics**

Since pharmacodynamic data using asparagine as a surrogate marker of efficacy were submitted as pivotal evidence of efficacy, these data are presented and discussed under the section on clinical efficacy. With regards to pharmacodynamic interactions with other medicinal products, the toxicity of vincristine may be additive with that of asparaginase if both agents are administered concomitantly. Therefore, vincristine should be given 3 to 24 hours before administration of r L asparaginase in order to minimise toxicity (see SmPC section 4.5). Within trial MC-ASP.5/ALL vincristine was given together with asparaginase on day 15 but no allergic reactions were observed on that day (see clinical safety). Potential interaction of vincristine and ASNase is adequately addressed in the RMP.

Concomitant use of glucocorticoids and/ or anticoagulants with asparaginase may increase the risk of a change in coagulation parameters (see section 4.4). This can promote tendency to bleeding (anticoagulants) or thrombosis (glucocorticoids). Caution is therefore needed when anticoagulants (e.g. coumarin, heparin, dipyridamole, acetylsalicylic acid or nonsteroidal anti-inflammatory medicinal products) or glucocorticoids are given at the same time (see SmPC section 4.5)..

Interactions with glucocorticoids (altered coagulation and increased risk of osteonecrosis) are adequately addressed in the risk management plan. Potential interactions with anticoagulants will also be closely monitored as important potential risks.

Inhibition of protein synthesis secondary to the asparaginase-induced depletion of asparagine has been shown to attenuate the cytotoxic effect of MTX which requires cell replication for its antineoplastic activity. This antagonism is observed if asparaginase is administered prior to or concurrently with methotrexate. Conversely, the antitumour effects of methotrexate are enhanced when asparaginase is administered 24 hours following methotrexate treatment. This regimen has been shown to reduce the gastrointestinal and haematological effects of methotrexate. (see SmPC section 4.5).

Laboratory in vitro and in vivo data indicate that the efficacy of high-dose cytarabine is reduced by prior administration of asparaginase. However, when asparaginase was given after cytarabine a synergistic effect was observed. This effect was most prominent with a treatment interval of about 120 hours (see SmPC section 4.5).

Schedule-dependent interactions with methotrexate and with Ara-C as well are adequately addressed in the RMP.

Asparaginase may increase the toxicity of other medicinal products through its effect on liver function, e.g. increased hepatotoxicity with potentially hepatotoxic medicines, increased toxicity of medicinal products metabolised by the liver or bound to plasma proteins and altered pharmacokinetics and pharmacodynamics of medicines bound to plasma proteins (see SmPC section 4.5). Potential interaction with other medicines where impaired liver metabolism could increase toxicity are covered in the RMP.

### 2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology data are considered sufficient and have been adequately reflected in the product information.

## 2.5. *Clinical efficacy*

### 2.5.1. Dose response study

No dose response study was submitted, please see the discussion on efficacy.

### 2.5.2. Main study

#### MC-ASP.5/ALL

***Methods***

This multicentre, randomised, active-controlled, double-blind, parallel-group phase III study was designed to assess the efficacy and safety of recombinant L-asparaginase in comparison to Asparaginase medac during treatment of children with de novo ALL.

***Study Participants***

Main inclusion Criteria

- Previously untreated T-lineage or precursor B-lineage ALL
- Patients had to have morphological proof of ALL and diagnosis had to be made from bone marrow morphology with ≥ 25% blasts
- Age ≥ 1 year and ≤ 18 years

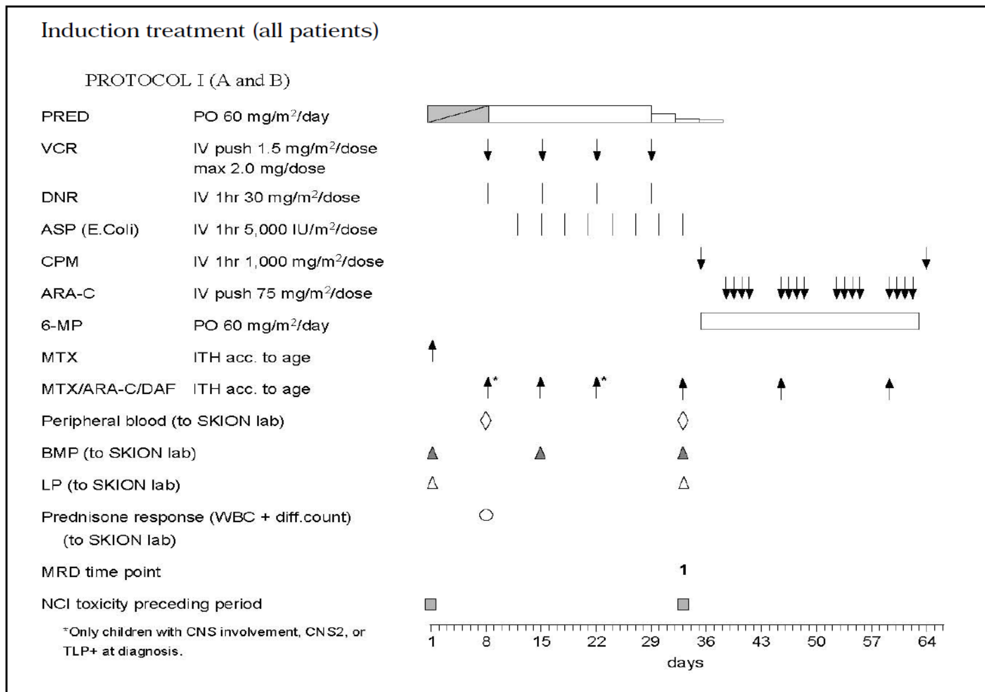
Main exclusion Criteria

- Mature B-lineage ALL
- Patients with secondary ALL
- General health status according to Karnofsky / Lansky score < 40%
- Pre-existing known coagulopathy (e.g. haemophilia) or pancreatitis
- Liver insufficiency

***Treatments***

Patients were treated according to the DCOG ALL 10 treatment protocol (briefly described below). Patients were randomised to receive eight IV doses of 5000 U/m² body surface area (BSA) of recombinant L-asparaginase or Asparaginase medac during part of the induction treatment. Dosage modifications were not allowed. Patients received additional chemotherapeutic and supportive agents as required by protocol DCOG ALL 10. There were no restrictions for the administration of subsequent therapies.

*Induction treatment section (PROTOCOL I [A and B]) of protocol DCOG ALL 10*




PRED = prednisone; VCR = vincristine; DNR = daunorubicin; ASP = asparaginase; CPM = cyclophosphamide;
ARA-C = cytarabine; 6-MP = 6-mercaptopurine; MTX = methotrexate; DAF = Diadreson F
I.TH. = intrathecal administration
BMP = bone marrow puncture; LP = lumbar puncture; MRD = minimal residual disease

Patients received their study drug infusions on days 12, 15, 18, 21, 24, 27, 30 and 33.

For the post-induction treatment, all patients were stratified into three risk groups (standard risk, medium risk, high risk [SR, MR, HR]) based on stratification criteria defined in the DCOG ALL 10 protocol. Pegaspargase (Oncaspar) was given to patients in the standard risk (SR) (one infusion of 2500 U/m² only) and medium risk (MR) (15 infusions of 2500 U/m² pegaspargase in Weeks 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29 of the intensification phase of the DCOG ALL 10) groups. High risk (HR) patients received four infusions of 10000 U/m² of the same asparaginase product as during induction treatment (on Days 22, 25, 29 and 32 of high risk blocks 1, 2, 4 and 5 and on Days 8, 11, 15 and 18 of protocol II, both of the DCOG ALL 10 protocol). Several chemotherapy agents were administered concomitantly throughout, as detailed in the DCOG ALL 10 protocol.

***Objectives***

To demonstrate non-inferiority of recombinant L-asparaginase to asparaginase medac with regard to complete depletion of serum asparagine (i.e. to show pharmacodynamic equivalence of both preparations).

***Outcomes/endpoints***

Primary endpoint:

Complete ASN depletion defined as ASN levels below the lower limit of quantitation (BLLQ) for at least three of the four scheduled time points (immediately before infusions nr 2, 4, 6, and 8).

Secondary endpoints

- hypersensitivity reactions to the first dose of asparaginase in the post-induction period. These also include silent inactivation of asparaginase activity, defined as asparaginase serum activity <20 U/L 3 days after administration of asparaginase to high risk patients

- incidence of pre-defined asparaginase-related AEs and overall AEs. These include; allergic reactions/ Liver parameters out of normal range (≥ CTCAE Grade III)/ Pancreatitis (> CTCAE Grade I)/ Haemorrhage or thromboembolism (≥ CTCAE Grade II)/ Neurotoxicity

- rate of complete ASN depletion in CSF on Day 33 of induction treatment

- trough levels of asparaginase activity in serum just before infusions nr 2, 4, 6 and 8 and in the post-induction period

- asparaginase activity levels in CSF during induction treatment phase A

- concentrations of amino acids ASN, ASP, GLN, and GLU in serum and CSF at defined time points during induction

- Trough levels of ASNase activity and ASN, ASP, GLN, and GLU levels in serum at defined time points during post-induction treatment

- anti-asparaginase antibodies in serum during repeated administration of ASNase

- CR rate and MRD status after induction treatment phase A

- Relapse rate, relapse-free survival (RFS) and EFS at end of study. 'Events' were relapse, death or premature termination of the protocol DCOG ALL 10. 'Relapse' was the recurrence of leukaemia after CR had been documented.

- number of patients in each risk group (standard risk, medium risk, high risk [SR, MR, HR]) who could complete their full course of asparaginase treatment as scheduled.

*Analytical methods*

*Asparaginase activity in various body fluids (plasma, serum, cerebrospinal fluid [CSF])*

Asparaginase activity was determined with a sensitive microplate reader-based method (AHA-assay).

CRS performed three ASNase evaluations of samples collected in clinical study MC-ASP.5/ALL:

− Original Method: ASNase values derived by applying the "Standard evaluation method plus an additional best curve fit evaluation" (SEM+BCF) as already applied in the original routine evaluation (referred to as original data)

− Method I: ASNase values derived by applying the strict "Standard evaluation method" (SEM) (dated: 04-Aug-2015).

− Method II: Inspection-induced re-evaluated and quality-controlled ASNase values applying the "Standard evaluation method plus an additional best curve fit evaluation" (SEM+BCF) as already applied in the Original Method evaluation (dated: 17-Oct-2014).

*Amino acids asparagine, glutamine, aspartic acid and glutamic acid (plasma, serum, CSF)*

The amino acids asparagine (ASN), aspartic acid (ASP), glutamine (GLN), and glutamic acid (GLU) are known to be influenced by asparaginase treatment and were therefore determined in serum or plasma and CSF. To avoid *ex vivo* cleavage of ASN by asparaginase, blood samples were processed according to a specific procedure. Within this procedure, the whole blood collected in vials without additive were immediately placed in an ice water bath and rapidly transferred to the laboratory where it was immediately centrifuged (600-800 G for 10 minutes). Serum and CSF samples for amino acid

determination were then deproteinised with 10% sulphosalicylic acid. Amino acid levels were measured using a liquid chromatography method with fluorescence detection.

*Anti-drug antibodies*

The assay is an indirect enzyme-linked immunosorbent assay (ELISA) using the drugs (Asparaginase medac or recombinant asparaginase) immobilised to the solid phase. The assay detects anti-asparaginase antibodies of the classes IgG and IgM simultaneously. ADAs in serum were determined at all time points during induction and post-induction treatment where blood sample collection was performed. For statistical analysis a blood sample was considered as anti-asparaginase antibody positive if at least one of both concentrations was 6.25 AU/mL or above. Otherwise, anti-asparaginase antibody tests were classified as negative.

***Sample size***

On the basis of published data on native asparaginase and the first results with recombinant asparaginase it was assumed that the success rate would be roughly 95% in the reference and experimental arms. In order to demonstrate statistically significant non-inferiority with a non-inferiority margin of -10% at a one-sided type I error of 2.5%, n = 94 patients per group were required within the Per Protocol Set to yield a power of 80% using the unconditional exact test statistic as outlined in the protocol. Assuming that 5% of the randomised patients did not qualify for the Per Protocol Set, 198 patients were to be enrolled for efficacy analysis.

***Randomisation***

Patients were randomised in a ratio of 1:1 with a randomisation stratified by centre using permuted blocks. The centre served as the stratification variable.

***Blinding (masking)***

The study was a double-blind clinical study. The investigators were blinded. However, the pharmacists were not, as they prepared the investigational products (IP) for administration to the patients. For high-risk patients in the post-induction phase, L-asparaginase treatment was also blinded.

***Statistical methods***

The confirmatory objective was to show that recombinant L-asparaginase is non-inferior to asparaginase medac with respect to the asparaginase-induced complete ASN depletion rate (cDR) in serum during the induction phase. The non-inferiority margin 'D' was pre-specified at -10 percentage points, i.e. a loss of $\Delta$=-10 percentage points on the absolute scale has been decided to represent a clinically relevant difference (inferiority) to be excluded by the evidence resulting from this trial. Having shown significant non-inferiority with respect to the primary efficacy parameter, a supplementary hypothesis system aimed to investigate further any potential significant clinical benefit with respect to hypersensitivity reactions (HSR) using a hierarchical approach.

*Analysis Populations*

- Safety Analysis Set included all randomised patients who received the IP at least once.
- Full Analysis Set included all randomised patients analysed according to ITT principles.
- Per Protocol Set consisted of all patients in the Full Analysis Set analysed in their actual treatment group, if IP was given and ASN serum levels were evaluable for at least three scheduled time points during induction phase.

Confirmatory statistical analysis of the primary efficacy parameter was performed within the Per Protocol Set and Full Analysis Set. Safety analyses were performed within the Safety Analysis Set.

For the primary efficacy endpoint, the unconditional exact test of non-inferiority for binomial differences based on restricted maximum likelihood estimates was applied. Statistical significance was claimed if the resulting p-value was less than the pre-specified significance level of 0.025. A two-sided 95% CI for the difference between complete ASN depletion rates (the rate for the recombinant asparaginase group minus the rate for the asparaginase medac group) was provided using the method by Chan and Zhang. Recombinant asparaginase was to be deemed to be not less effective than asparaginase medac if the lower limit of this CI was greater than -10%.

***Results***

**Participant flow**

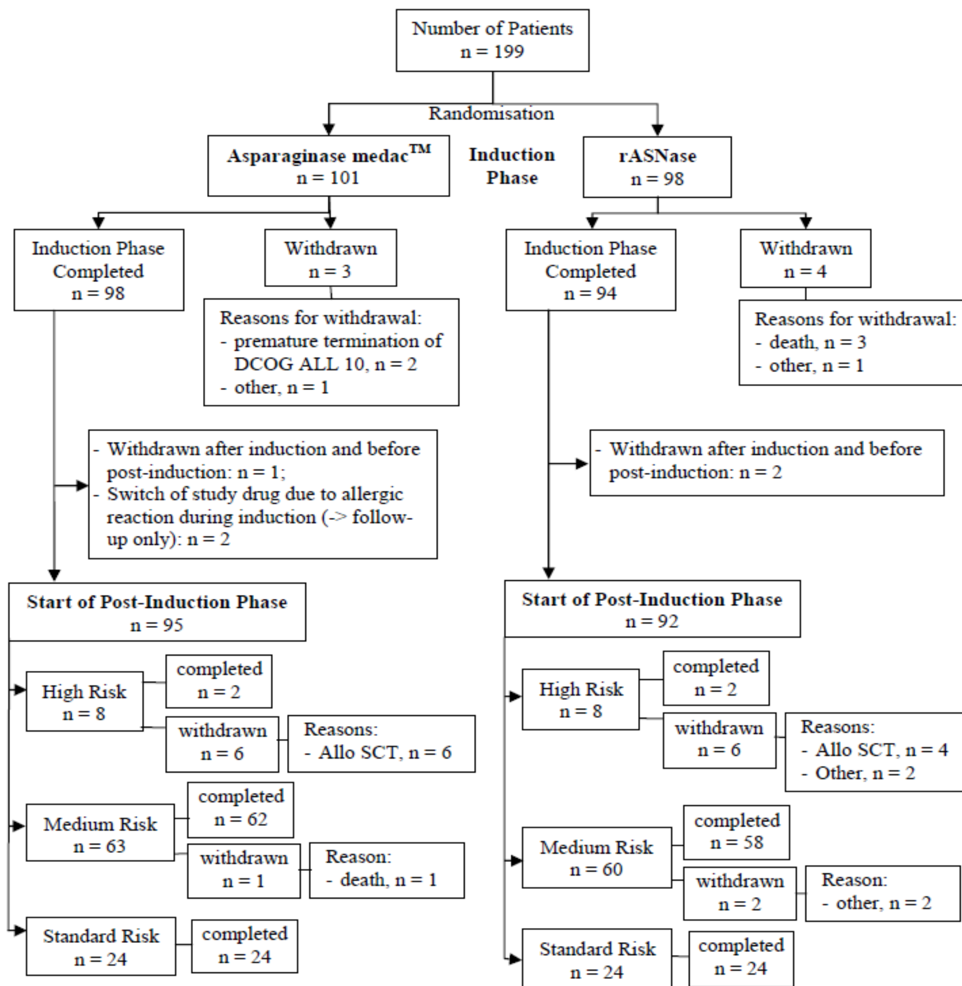



**Figure 1: Disposition of Patients in MC-ASP.5/ALL**

## Recruitment

The first patient was randomised for study participation on 29-Oct-2008. The last visit of the last patient was performed on 17-Feb-2012. Approximately 30% of study subjects were enrolled at one site in Rotterdam. All other sites in the study were in the Netherlands.

## Conduct of the study

*Protocol Deviations*

Of the 199 patients included in this clinical study (Full Analysis Set), 59 patients (29.6%) showed major protocol deviations. Of these, 26 (25.7%) patients received Asparaginase medac (33.7%) and 33 patients received recombinant L-asparaginase. Violation of inclusion or exclusion criteria at study entry occurred in 11 patients (5.5%), six patients receiving Asparaginase medac and five patients receiving recombinant L-asparaginase. Ten patients violated inclusion criterion no. 2. Other major deviations include missing evaluations for serum samples, time schedule deviations and treatment deviations (e.g. study drug not being administered).

## Baseline data

Demographic and other baseline characteristics

The two treatment groups were comparable regarding the demographic data. The trial enrolled an adequate number of patients to each paediatric age subgroup. Infants were not enrolled in this study.

**Table 9: Summary of Demographic Data – Safety Analysis Set (MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Age [years] | | | |
| Mean (SD) | 5.9 (4.5) | 6.7 (4.6) | 6.3 (4.5) |
| Median (Q1, Q3) | 4.0 (2.0, 9.0) | 5.0 (3.0, 10.0) | 5.0 (2.0, 9.0) |
| Min, Max | 1, 16 | 1, 17 | 1, 17 |
| Age group | | | |
| 1-<=2 yrs | 32 ( 31.7%) | 21 ( 21.4%) | 53 ( 26.6%) |
| >2-<=6 yrs | 33 ( 32.7%) | 35 ( 35.7%) | 68 ( 34.2%) |
| >6-<=12 yrs | 23 ( 22.8%) | 29 ( 29.6%) | 52 ( 26.1%) |
| >12-<18 yrs | 13 ( 12.9%) | 13 ( 13.3%) | 26 ( 13.1%) |
| Sex | | | |
| female | 48 ( 47.5%) | 44 ( 44.9%) | 92 ( 46.2%) |
| male | 53 ( 52.5%) | 54 ( 55.1%) | 107 ( 53.8%) |
| Body Weight [kg] | | | |
| Mean (SD) | 25.7 (16.3) | 27.4 (16.3) | 26.6 (16.3) |
| Median (Q1, Q3) | 18.0 (14.4, 32.0) | 22.0 (15.9, 33.4) | 20.0 (14.8, 33.0) |
| Min, Max | 7, 71 | 9, 90 | 7, 90 |
| Body Height [cm] | | | |
| Mean (SD) | 118.8 (29.3) | 123.0 (28.0) | 120.9 (28.6) |
| Median (Q1, Q3) | 108.0 (96.0, 142.0) | 118.5 (98.0, 141.0) | 115.0 (97.0, 142.0) |
| Min, Max | 72, 191 | 75, 185 | 72, 191 |
| BSA [m²] | | | |
| Mean (SD) | 0.91 (0.39) | 0.95 (0.38) | 0.93 (0.38) |
| Median (Q1, Q3) | 0.73 (0.62, 1.12) | 0.85 (0.66, 1.14) | 0.79 (0.63, 1.14) |
| Min, Max | 0.4, 1.9 | 0.4, 2.1 | 0.4, 2.1 |
| Ethnicity | | | |
| Asian | 4 ( 4.0%) | 2 ( 2.0%) | 6 ( 3.0%) |
| Caucasian | 93 ( 92.1%) | 93 ( 94.9%) | 186 ( 93.5%) |
| Other | 4 ( 4.0%) | 3 ( 3.1%) | 7 ( 3.5%) |

Source: Listing 16.2.4D

**Table 10: Summary of Disease Characteristics at Baseline – Full Analysis Set (MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Immunophenotype | | | |
| Common ALL | 58 ( 57.4%) | 62 ( 63.3%) | 120 ( 60.3%) |
| Pre-B-ALL | 26 ( 25.7%) | 21 ( 21.4%) | 47 ( 23.6%) |
| T-ALL | 14 ( 13.9%) | 12 ( 12.2%) | 26 ( 13.1%) |
| Pre-Pre-B-ALL | 3 ( 3.0%) | 1 ( 1.0%) | 4 ( 2.0%) |
| MPAL: T/myeloid | 0 (0%) | 1 ( 1.0%) | 1 ( 0.5%) |
| ND | 0 (0%) | 1 ( 1.0%) | 1 ( 0.5%) |
| Genetics | | | |
| No aberration or not yet defined | 46 ( 45.5%) | 45 ( 45.9%) | 91 ( 45.7%) |
| Hyperdiploid | 28 ( 27.7%) | 28 ( 28.6%) | 56 ( 28.1%) |
| TEL/AML1 | 15 ( 14.9%) | 16 ( 16.3%) | 31 ( 15.6%) |
| TEL/AML1; Hyperdiploid | 1 ( 1.0%) | 1 ( 1.0%) | 2 ( 1.0%) |
| BCR/ABL | 1 ( 1.0%) | 0 (0%) | 1 ( 0.5%) |
| MLL-AF4 | 1 ( 1.0%) | 0 (0%) | 1 ( 0.5%) |
| Other | 10 ( 9.9%) | 11 ( 11.2%) | 21 ( 10.6%) |

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Peripheral blood: blasts [%] | | | |
| Nmiss | 1 | 0 | 1 |
| Mean (SD) | 49.24 (31.08) | 40.74 (31.67) | 45.03 (31.58) |
| Median (Q1, Q3) | 57.50 (22.00, 74.00) | 34.50 (13.00, 68.00) | 48.00 (17.00, 73.00) |
| Min, Max | 0.0, 96.0 | 0.0, 96.6 | 0.0, 96.6 |
| Peripheral blood: WBC [x$10^9$/L] | | | |
| Nmiss | 1 | 0 | 1 |
| Mean (SD) | 57.59 (125.69) | 33.67 (75.67) | 45.75 (104.41) |
| Median (Q1, Q3) | 12.95 (5.80, 38.30) | 11.60 (4.90, 24.50) | 12.30 (5.70, 31.20) |
| Min, Max | 0.1, 694.6 | 1.1, 573.0 | 0.1, 694.6 |
| CSF: blasts | | | |
| No | 38 ( 37.6%) | 51 ( 52.0%) | 89 ( 44.7%) |
| Yes | 63 ( 62.4%) | 46 ( 46.9%) | 109 ( 54.8%) |
| NK | 0 (0%) | 1 ( 1.0%) | 1 ( 0.5%) |
| Bone marrow biopsy/aspirate: marrow blasts [%] | | | |
| Nmiss | 8 | 6 | 14 |
| Mean (SD) | 86.94 (12.99) | 84.89 (14.72) | 85.92 (13.87) |
| Median (Q1, Q3) | 91.00 (83.00, 95.00) | 90.10 (80.50, 95.00) | 90.80 (83.00, 95.00) |
| Min, Max | 28.0, 100.0 | 26.0, 98.8 | 26.0, 100.0 |
| Risk group at start of post-induction | | | |
| Standard risk | 24 ( 23.8%) | 24 ( 24.5%) | 48 ( 24.1%) |
| Medium risk | 63 ( 62.4%) | 60 ( 61.2%) | 123 ( 61.8%) |
| High risk | 8 ( 7.9%) | 8 ( 8.2%) | 16 ( 8.0%) |
| NA | 6 ( 5.9%) | 6 ( 6.1%) | 12 ( 6.0%) |

Source: Listing 16.2.4E, Listing 16.2.6H

**Numbers analysed**

**Table 11: Number of patients analysed (Study MC-ASP.5/ALL)**

| | ASNase Medac | rASNase | Total |
|---|---|---|---|
| Number of patients<br>Full Analysis set | <br>101 | <br>98 | <br>199 |
| Per protocol set | 97 | 93 | 190 |
| Safety Analysis Set | 101 | 97 | 198 |

**Outcomes and estimation**

Primary endpoint

*Complete Asparagine depletion in Serum during Induction Phase*

**Table 12: Summary of Complete Asparagine Depletion in Serum during Induction Phase, Full Analysis Set, Per Protocol Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Full Analysis Set** | | | |
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Complete ASN depletion | | | |
| Yes | 95 ( 94.1%) | 93 ( 94.9%) | 188 ( 94.5%) |
| No | 2 ( 2.0%) | 2 ( 2.0%) | 4 ( 2.0%) |
| n.e. | 4 ( 4.0%) | 3 ( 3.1%) | 7 ( 3.5%) |
| Difference[a] (rASNase - ASNase medac) | 0.8% | | |
| 95% CI[b] | [-6.25%; 8.04%] | | |
| p-value[c] | 0.0028 | | |
| **Per Protocol Set** | | | |
| Number of patients | 97 (100.0%) | 93 (100.0%) | 190 (100.0%) |
| Complete ASN depletion | | | |
| Yes | 95 ( 97.9%) | 91 ( 97.8%) | 186 ( 97.9%) |
| No | 2 ( 2.1%) | 2 ( 2.2%) | 4 ( 2.1%) |
| Difference[a] (rASNase - ASNase medac) | -0.1% | | |
| 95% CI[b] | [-5.67%; 5.31%] | | |
| p-value[c] | 0.0019 | | |

Source: Table 14.2.1A, Table 14.2.1C, Listing 16.2.6A, Listing 16.2.6B
[a] If ASN depletion is not evaluable (n.e.), patient will be considered to be not completely depleted.
[b] unconditional exact confidence interval based on Chan and Zhang
[c] unconditional exact test of non-inferiority for binomial differences based on restricted maximum likelihood estimates

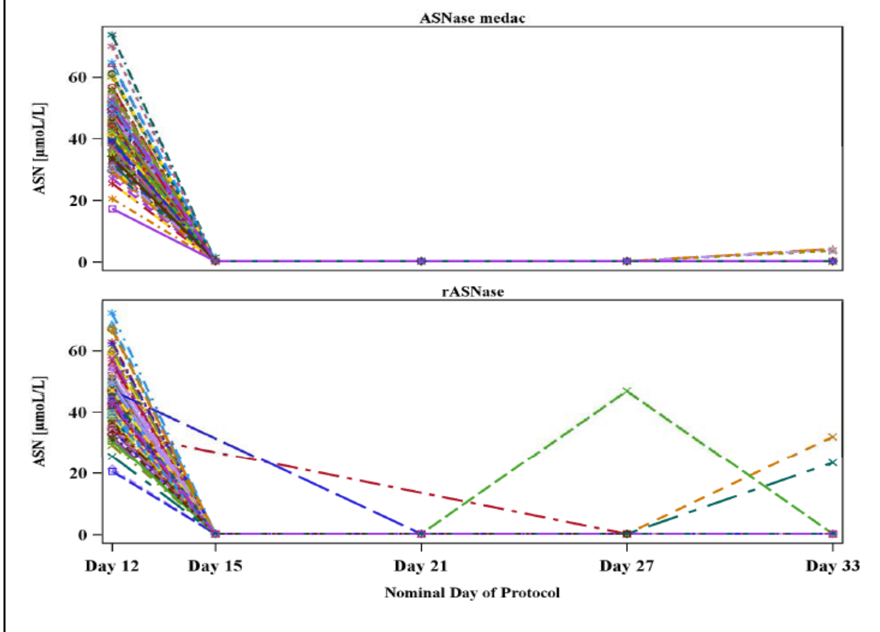



**Figure 2: Linear plot of individual ASN concentrations in serum during induction phase (Full Analysis Set) (study MC-ASP.5/ALL)**

**Table 13: Absolute values [µmol/L] and relative reductions from baseline [%] of ASN concentrations in serum during induction phase (Full Analysis Set) (study MC-ASP.5/ALL)**

**Table 14.2.1B: Absolute values [µmol/L] and relative reductions from baseline [%] of ASN concentrations in serum during induction phase (Full Analysis Set)**

(Page 1 of 2)

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Day 12 | | | |
| Absolute values | | | |
| N | 101 | 96 | 197 |
| Mean (SD) | 43.62 (11.56) | 44.49 (9.84) | 44.04 (10.74) |
| 95% CI for Mean | [41.34 - 45.90] | [42.49 - 46.48] | [42.53 - 45.55] |
| Median (Q1, Q3) | 43.69 (34.94, 51.04) | 43.87 (38.32, 49.47) | 43.72 (36.49, 49.74) |
| Min - Max | 17.31 - 73.77 | 20.57 - 72.31 | 17.31 - 73.77 |
| Day 15 | | | |
| Absolute values | | | |
| N | 99 | 94 | 193 |
| Mean (SD) | 0.30 (0.13) | 0.29 (0.00) | 0.29 (0.09) |
| 95% CI for Mean | [0.27 - 0.32] | [0.29 - 0.29] | [0.28 - 0.31] |
| Median (Q1, Q3) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 1.57 | 0.29 - 0.29 | 0.29 - 1.57 |
| Relative reduction | | | |
| N | 99 | 94 | 193 |
| Mean (SD) | 99.3 (0.5) | 99.3 (0.2) | 99.3 (0.4) |
| 95% CI for Mean | [99.1, 99.4] | [99.3, 99.4] | [99.2, 99.3] |
| gMean | 99.2 | 99.3 | 99.3 |
| 95% CI for gMean | [99.1, 99.4] | [99.3, 99.4] | [99.2, 99.3] |
| Median (Q1, Q3) | 99.4 (99.2, 99.4) | 99.3 (99.3, 99.4) | 99.4 (99.2, 99.4) |
| Min - Max | 94.8 - 99.6 | 98.6 - 99.6 | 94.8 - 99.6 |

### *Secondary endpoints*

*Trough levels of asparaginase activity in serum during induction phase*

**Table 14: Descriptive Statistics of Baseline Values and Trough Levels of Asparaginase Activity [U/L] in Serum during Induction Phase Full Analysis Set (study MC-ASP.5/ALL)**

| Day of protocol | | ASNase medac | rASNase | Total |
|---|---|---|---|---|
| Day 12 | N | 101 | 96 | 197 |
| | gMean (gCV [%]) | 1.25 (0.00) | 1.25 (0.00) | 1.25 (0.00) |
| | 95% CI for gMean | [1.25, 1.25] | [1.25, 1.25] | [1.25, 1.25] |
| | Median (Q1, Q3) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) |
| | Min - Max | 1.25 - 1.25 | 1.25 - 1.25 | 1.25 - 1.25 |
| Day 15 | N | 99 | 92 | 191 |
| | gMean (gCV [%]) | 146.65 (56.11) | 159.87 (65.10) | 152.88 (60.55) |
| | 95% CI for gMean | [132.12, 162.78] | [141.36, 180.82] | [141.16, 165.57] |
| | Median (Q1, Q3) | 144.59 (96.48, 221.58) | 167.06 (114.72, 257.26) | 150.85 (104.22, 243.35) |
| | Min - Max | 35.35 - 401.72 | 28.14 - 524.44 | 28.14 - 524.44 |
| Day 21 | N | 95 | 94 | 189 |
| | gMean (gCV [%]) | 154.15 (91.99) | 156.84 (86.29) | 155.48 (88.86) |
| | 95% CI for gMean | [131.42, 180.81] | [134.61, 182.73] | [139.36, 173.47] |
| | Median (Q1, Q3) | 167.15 (109.81, 248.63) | 175.11 (115.22, 268.07) | 171.03 (112.74, 261.03) |
| | Min - Max | 1.25 - 584.25 | 3.69 - 696.33 | 1.25 - 696.33 |
| Day 27 | N | 92 | 89 | 181 |
| | gMean (gCV [%]) | 176.41 (67.14) | 149.73 (97.42) | 162.74 (82.70) |
| | 95% CI for gMean | [155.47, 200.16] | [126.06, 177.85] | [146.39, 180.92] |
| | Median (Q1, Q3) | 180.36 (131.72, 262.38) | 176.57 (99.88, 257.22) | 177.23 (108.44, 257.78) |
| | Min - Max | 18.37 - 750.08 | 1.25 - 739.53 | 1.25 - 750.08 |
| Day 33 | N | 95 | 93 | 188 |
| | gMean (gCV [%]) | 153.73 (158.19) | 125.49 (186.18) | 139.04 (172.10) |
| | 95% CI for gMean | [122.38, 193.11] | [97.54, 161.44] | [117.44, 164.61] |
| | Median (Q1, Q3) | 179.52 (117.72, 286.93) | 173.25 (96.24, 250.78) | 178.44 (102.78, 260.75) |
| | Min - Max | 1.25 - 1980.94 | 1.25 - 697.80 | 1.25 - 1980.94 |

Source: Table 14.2.2A, Listing 16.2.6A

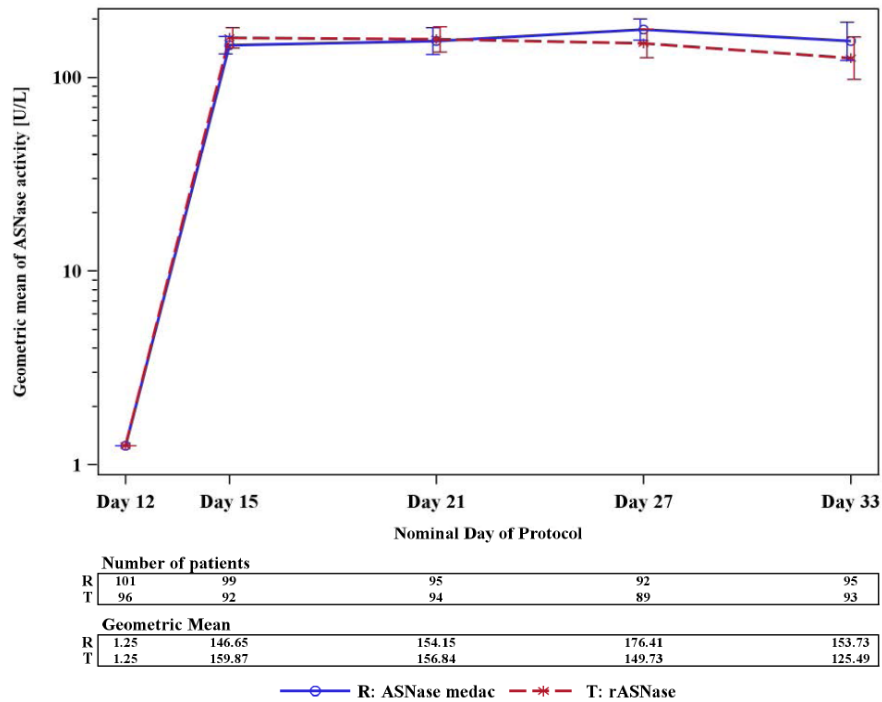



**Figure 3: Plot of geometric mean and 95% CI of baseline values and trough levels of ASNase activity in serum during induction phase – Full Analysis set (study MC-ASP.5/ALL)**

The median levels of asparaginase activity in the asparaginase medac group changed from 1.25 U/L at baseline (Day 12) to median trough levels of 144.59 U/L on Day 15, 167.15 U/L on Day 21, 180.36 U/L on Day 27 and 179.52 U/L on Day 33. In the recombinant asparaginase group, the median levels of asparaginase activity changed from 1.25 U/L at baseline (Day 12) to median trough levels of 167.06 U/L on Day 15, 175.11 U/L on Day 21, 176.57 U/L on Day 27 and 173.25 U/L on Day 33.

**Figure 14.2.2E: Plot of geometric mean and 95% CI of baseline values and trough levels of ASNase activity in serum during induction phase stratified by age group (Per Protocol Set)**

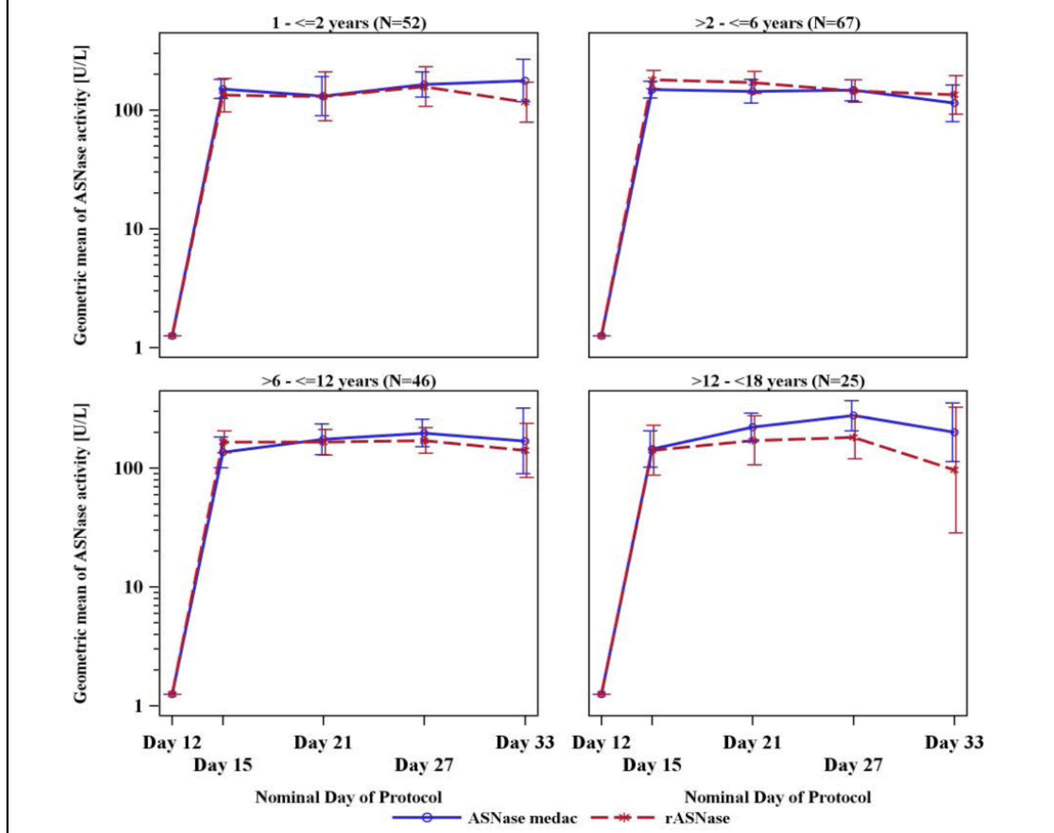



**Figure 4: Plot of geometric mean and 95% CI of baseline values and trough levels of ASNase activity in serum during induction phase stratified by age group – Per protocol set (study MC-ASP.5/ALL)**

Following the request of recalculating asparaginase data derived using the "standard evaluation method" (i.e. without using an additional best curve fit evaluation), the results of three different evaluation methods (including the original method) were reported and are presented below.

The number of non-valid samples was considerably increased when applying the more stringent Method I, compared to both the original Method and Method II. In total, the percentage of patients providing a complete set of ASNase values after start of ASNase-infusion is 87.9%, 87.9% and 73.4% when respectively applying the Original Method, Method II and the more stringent Method I. One third of the number of non-valid samples (n=59) produced with Method I occurred on Day 12 prior to infusion of any ASNase preparation (baseline). Considering only those samples providing valid results, the median differences in ASNase activity values in serum as well as the quartiles and 5% and 95% quantiles equalled zero between Method II and Method I. Between the data of the Original Method and the re-evaluated data according to Method II, the median differences as well as the quartiles were zero. Similar results were observed for the comparison between the data of Method I and the Original Method. However, when the means are taken into consideration, differences between initial and method I data were larger than those reported among initial and method II data.

The ASNase activity data obtained with method I of re-evaluation are presented below.

**Table 15: Descriptive statistics of trough levels of ASNase activity [U/L] in serum during induction phase (Evaluation method I)**

| Day of protocol | | ASNase medac | rASNase |
|---|---|---|---|
| Day 15 | N | 86 | 81 |
| | Median (Q1, Q3) | 152.15 (105.17, 242.49) | 168.21 (119.15, 259.69) |
| | Min - Max | 47.46 - 389.52 | 28.14 - 524.44 |
| | N | 86 | 81 |
| | gMean (gCV [%]) | 156.74 (49.50) | 165.01 (63.55) |
| Day 21 | N | 82 | 83 |
| | Median (Q1, Q3) | 174.04 (122.84, 262.58) | 180.03 (116.28, 265.53) |
| | Min - Max | 1.25 - 584.69 | 3.69 - 696.33 |
| | N | 82 | 83 |
| | gMean (gCV [%]) | 161.29 (92.23) | 160.89 (84.63) |
| Day 27 | N | 82 | 82 |
| | Median (Q1, Q3) | 182.20 (132.56, 253.85) | 176.90 (96.89, 244.78) |
| | Min - Max | 18.37 - 747.61 | 1.25 - 739.53 |
| | N | 82 | 82 |
| | gMean (gCV [%]) | 178.65 (63.82) | 149.64 (98.93) |
| Day 33 | N | 82 | 80 |
| | Median (Q1, Q3) | 180.95 (122.22, 286.93) | 184.18 (101.70, 265.44) |
| | Min - Max | 1.25 - 1980.94 | 1.25 - 697.80 |
| | N | 82 | 80 |
| | gMean (gCV [%]) | 168.50 (132.85) | 132.89 (190.82) |
| *Data source: Table 14.2.2A, Section 9.6, Addendum 04 of MC-ASP.5/ALL clinical study report (dated: 10-Aug-2015)* | | | |

*Concentrations of ASP, GLN and GLU in serum during induction phase*

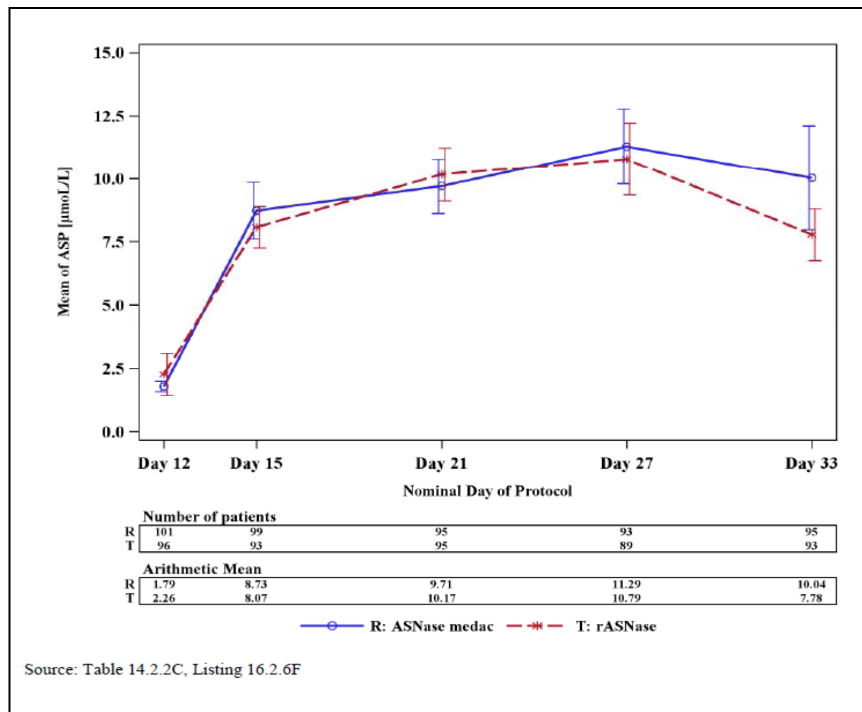



**Figure 5: Plot of Arithmetic Mean and 95% CI of Concentrations of Aspartic Acid in Serum during Induction Phase – Full-Analysis Set (study MC-ASP.5/ALL)**

Median concentrations of ASP in serum at baseline and before administrations of IP during induction phase were comparable between treatment groups. Median ASP levels increased about four- to six-fold compared to baseline levels and were still four-fold above baseline on Day 33.

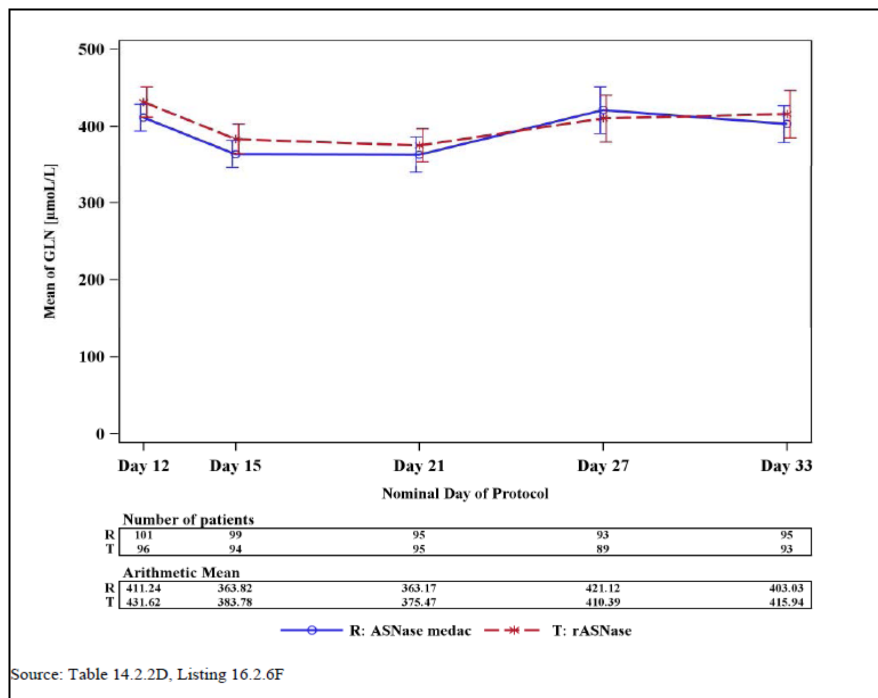



**Figure 6: Plot of Arithmetic Mean and 95% CI of Glutamine in Serum during Induction Phase - Full-Analysis Set (study MC-ASP.5/ALL)**

Mean concentrations of GLN in serum at baseline were comparable between treatment groups. They declined only slightly from pre-dose levels of 421 µmoL/L to 374 µmoL/L on Day 15 and 369 µmoL/L on Day 21, but returned to 416 µmoL/L on Day 27 and 409 µmoL/L on Day 33. Whereas ASN is completely depleted in serum in the majority of patients, GLN serum level are only marginally and temporarily influenced by asparaginase treatment. Nevertheless, a continuous turnover of GLN takes place during asparaginase treatment as reflected by the increase of the cleavage product GLU.

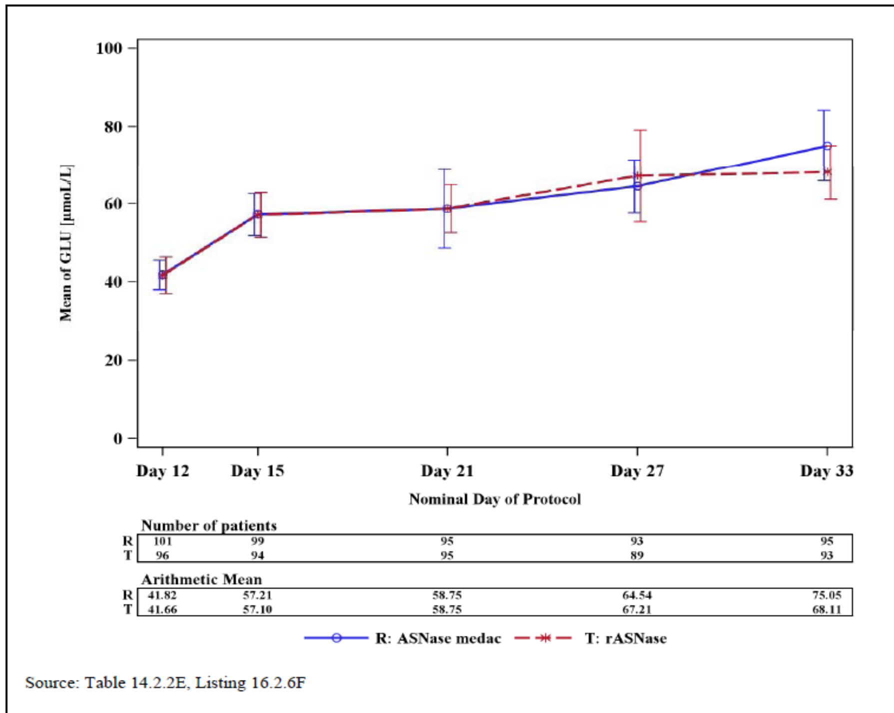



**Figure 7: Plot of Arithmetic Mean and 95% CI of Concentrations of Glutamic Acid in Serum during Induction Phase – Full-Analysis Set (study MC-ASP.5/ALL)**

*Concentrations of Asparagine in Cerebrospinal Fluid during Induction Phase*

**Table 16: Absolute values [µmoL/L] and relative reductions from baseline [%] of ASN concentrations in CSF during induction phase (Full Analysis Set) (study MC-ASP.5/ALL)**

**Table 14.2.3A: Absolute values [µmoL/L] and relative reductions from baseline [%] of ASN concentrations in CSF during induction phase (Full Analysis Set)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Day 1 | | | |
| Absolute values | | | |
| N | 101 | 94 | 195 |
| Mean (SD) | 3.99 (1.18) | 4.14 (1.38) | 4.06 (1.28) |
| 95% CI for Mean | [3.75 - 4.22] | [3.86 - 4.42] | [3.88 - 4.24] |
| Median (Q1, Q3) | 3.96 (3.23, 4.51) | 4.01 (3.36, 4.55) | 3.99 (3.34, 4.51) |
| Min - Max | 0.29 - 8.73 | 1.64 - 13.54 | 0.29 - 13.54 |
| Day 33 | | | |
| Absolute values | | | |
| N | 94 | 83 | 177 |
| Mean (SD) | 0.41 (0.62) | 0.40 (1.08) | 0.41 (0.87) |
| 95% CI for Mean | [0.28 - 0.54] | [0.17 - 0.64] | [0.28 - 0.54] |
| Median (Q1, Q3) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 4.75 | 0.29 - 10.15 | 0.29 - 10.15 |
| Relative reduction | | | |
| N | 94 | 83 | 177 |
| Mean (SD) | 71.9 (171.2) | 89.0 (33.2) | 79.9 (126.8) |
| 95% CI for Mean | [36.9, 107.0] | [81.7, 96.2] | [61.1, 98.7] |
| Median (Q1, Q3) | 92.8 (91.0, 93.7) | 93.0 (91.5, 93.8) | 92.9 (91.5, 93.7) |
| Min - Max | -1563.7 - 96.7 | -209.5 - 97.9 | -1563.7 - 97.9 |

Source: Listing 16.2.6G

*Complete Asparagine Depletion in Cerebrospinal Fluid on Day 33 of Induction Phase*

**Table 17: Summary of Complete Asparagine Depletion in Cerebrospinal Fluid on Day 33 of Induction Phase – Full Analysis Set, Per Protocol Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Full Analysis Set** | | | |
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Complete ASN depletion | | | |
| Yes | 88 ( 87.1%) | 82 ( 83.7%) | 170 ( 85.4%) |
| No | 6 ( 5.9%) | 1 ( 1.0%) | 7 ( 3.5%) |
| n.e. | 7 ( 6.9%) | 15 ( 15.3%) | 22 ( 11.1%) |
| Difference[a] (rASNase - ASNase medac) | -3.5% | | |
| 95% CI[b] | [-13.67%; 6.58%] | | |
| **Per Protocol Set** | | | |
| Number of patients | 97 (100.0%) | 93 (100.0%) | 190 (100.0%) |
| Complete ASN depletion | | | |
| Yes | 88 ( 90.7%) | 81 ( 87.1%) | 169 ( 88.9%) |
| No | 6 ( 6.2%) | 1 ( 1.1%) | 7 ( 3.7%) |
| n.e. | 3 ( 3.1%) | 11 ( 11.8%) | 14 ( 7.4%) |
| Difference[a] (rASNase - ASNase medac) | -3.6% | | |
| 95% CI[b] | [-13.23%; 5.65%] | | |

Source: Listing 16.2.6G, Listing 16.2.6B
[a] If ASN depletion is not evaluable (n.e.), patient will be considered to be not completely depleted.
[b] unconditional exact confidence interval based on Chan and Zhang

*Asparaginase Activity in Cerebrospinal Fluid during Induction Phase*

**Table 18: Descriptive Statistics of Asparaginase Activity [U/L] in Cerebrospinal Fluid on Day 1 and Day 33 of Induction Phase– Full Analysis Set (study MC-ASP.5/ALL)**

| Day of protocol | | ASNase medac | rASNase | Total |
|---|---|---|---|---|
| Day 1 | N | 101 | 94 | 195 |
| | gMean (gCV [%]) | 1.25 (0.00) | 1.25 (0.00) | 1.25 (0.00) |
| | 95% CI for gMean | [1.25, 1.25] | [1.25, 1.25] | [1.25, 1.25] |
| | Median (Q1, Q3) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) |
| | Min - Max | 1.25 - 1.25 | 1.25 - 1.25 | 1.25 - 1.25 |
| Day 33 | N | 94 | 82 | 176 |
| | gMean (gCV [%]) | 1.34 (55.74) | 1.27 (10.99) | 1.31 (40.27) |
| | 95% CI for gMean | [1.21, 1.50] | [1.24, 1.30] | [1.23, 1.38] |
| | Median (Q1, Q3) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) | 1.25 (1.25, 1.25) |
| | Min - Max | 1.25 - 106.24 | 1.25 - 3.37 | 1.25 - 106.24 |

Source: Table 14.2.4A, Listing 16.2.6G

The median asparaginase activity in CSF on Days 1 and 33 was below Lower limit of quantification (LLoQ) in both treatment groups. Only three samples (two in the reference group and one in the recombinant asparaginase group) contained measurable amounts (>LLoQ) of asparaginase activity on Day 33 of induction treatment. This confirms results of other study groups that this protein is not able to cross the blood-brain barrier in measurable amounts.

*Concentrations of Aspartic Acid, Glutamine and Glutamic Acid in Cerebrospinal Fluid during Induction Phase*

The median concentrations of ASP and GLU in CSF were below LLoQ on Days 1 and 33 of induction phase in both treatment groups. Mean estimates however showed similar and small reductions in GLU concentrations in each arm but little if any change in ASP concentrations. The mean and median concentrations of GLN in CSF changed little during induction and were comparable between treatment groups.

*Asparaginase Activity and Trough Concentrations of Asparagine, Aspartic Acid, Glutamine and Glutamic Acid in Serum during Post-Induction Phase*

During post-induction, blood sampling for the determination of asparaginase activity was performed on Day 1 and Day 8 of protocol IV for SR patients and on Day 1 and Day 15 of the intensification phase for MR patients. For HR patients, blood sampling was performed on Day 22, Day 25 and Day 32 of HR blocks 1, 2, 4 and 5 as well as on Day 8, 11 and 18 of protocol II.

**Table 19: Descriptive Statistics of Asparaginase Activity [U/L] in Serum during Post-Induction Phase (High Risk Blocks 1 and 2) – High Risk Patients - Full Analysis Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **HR block 1** | | | |
| Day 22 | | | |
| N | 7 (100%) | 8 (100%) | 15 (100%) |
| ASNase activity BLLQ | 7 (100%) | 8 (100%) | 15 (100%) |
| Day 25 | | | |
| N | 5 (100%) | 7 (100%) | 12 (100%) |
| ASNase activity BLLQ | 2 ( 40%) | 0 (0%) | 2 ( 17%) |
| ASNase activity above LLoQ | 3 ( 60%) | 7 (100%) | 10 ( 83%) |
| gMean (gCV [%]) | 271.97 (10.24) | 210.06 (89.93) | 226.98 (71.51) |
| Median (Q1, Q3) | 257.10 (255.71, 306.01) | 229.06 (111.12, 435.77) | 256.41 (166.11, 354.94) |
| Min - Max | 255.71 - 306.01 | 58.62 - 470.70 | 58.62 - 470.70 |
| Day 32 | | | |
| N | 4 (100%) | 6 (100%) | 10 (100%) |
| ASNase activity BLLQ | 2 ( 50%) | 0 (0%) | 2 ( 20%) |
| ASNase activity above LLoQ | 2 ( 50%) | 6 (100%) | 8 ( 80%) |
| gMean (gCV [%]) | 155.24 (20.80) | 160.75 (79.53) | 159.35 (65.44) |
| Median (Q1, Q3) | 156.89 (134.21, 179.56) | 157.52 (89.07, 234.80) | 156.89 (95.60, 223.86) |
| Min - Max | 134.21 - 179.56 | 79.64 - 476.45 | 79.64 - 476.45 |

Source: Table 14.2.5C, Listing 16.2.6E

continued

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **HR block 2** | | | |
| Day 22 | | | |
| N | 4 (100%) | 7 (100%) | 11 (100%) |
| ASNase activity BLLQ | 4 (100%) | 7 (100%) | 11 (100%) |
| Day 25 | | | |
| N | 2 (100%) | 6 (100%) | 8 (100%) |
| ASNase activity BLLQ | 1 ( 50%) | 0 (0%) | 1 ( 13%) |
| ASNase activity above LLoQ | 1 ( 50%) | 6 (100%) | 7 ( 88%) |
| gMean (gCV [%]) | n.c. | 214.19 (53.57) | 187.43 (63.08) |
| Median (Q1, Q3) | n.c. | 205.12 (138.49, 274.01) | 185.18 (124.22, 274.01) |
| Min - Max | n.c. | 124.22 - 491.54 | 84.14 - 491.54 |
| Day 32 | | | |
| N | 3 (100%) | 6 (100%) | 9 (100%) |
| ASNase activity BLLQ | 1 ( 33%) | 0 (0%) | 1 ( 11%) |
| ASNase activity above LLoQ | 2 ( 67%) | 6 (100%) | 8 ( 89%) |
| gMean (gCV [%]) | 145.98 (2.90) | 94.55 (101.14) | 105.40 (85.00) |
| Median (Q1, Q3) | 146.01 (143.02, 149.00) | 66.91 (58.63, 200.74) | 108.12 (59.62, 174.87) |
| Min - Max | 143.02 - 149.00 | 39.35 - 347.73 | 39.35 - 347.73 |

Source: Table 14.2.5C, Listing 16.2.6E
n.c.: not calculated due to single observation

**Table 20: Descriptive Statistics of Concentrations of Asparagine [µmol/L] in Serum during Post- Induction Phase (High Risk Blocks 1 and 2) High Risk Patients - Full Analysis Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **HR block 1** | | | |
| Day 22 | | | |
| N | 7 | 8 | 15 |
| Mean (SD) | 28.64 (5.13) | 31.20 (9.70) | 30.00 (7.75) |
| Median (Q1, Q3) | 29.88 (23.71, 33.95) | 29.68 (25.58, 34.92) | 29.88 (24.51, 33.95) |
| Min - Max | 20.91 - 34.63 | 18.98 - 50.25 | 18.98 - 50.25 |
| Day 25 | | | |
| N | 5 | 7 | 12 |
| Mean (SD) | 11.02 (15.42) | 0.29 (0.00) | 4.76 (10.82) |
| Median (Q1, Q3) | 0.29 (0.29, 20.51) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 33.73 | 0.29 - 0.29 | 0.29 - 33.73 |
| Day 32 | | | |
| N | 4 | 6 | 10 |
| Mean (SD) | 4.28 (6.50) | 0.29 (0.00) | 1.88 (4.28) |
| Median (Q1, Q3) | 1.47 (0.29, 8.27) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 13.88 | 0.29 - 0.29 | 0.29 - 13.88 |
| **HR block 2** | | | |
| Day 22 | | | |
| N | 4 | 7 | 11 |
| Mean (SD) | 35.50 (20.88) | 32.25 (7.48) | 33.43 (12.93) |
| Median (Q1, Q3) | 25.94 (23.80, 47.21) | 33.43 (25.34, 37.45) | 27.71 (24.94, 37.45) |
| Min - Max | 23.42 - 66.70 | 24.94 - 44.81 | 23.42 - 66.70 |
| Day 25 | | | |
| N | 2 | 6 | 8 |
| Mean (SD) | 11.11 (15.30) | 0.29 (0.00) | 2.99 (7.65) |
| Median (Q1, Q3) | 11.11 (0.29, 21.93) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 21.93 | 0.29 - 0.29 | 0.29 - 21.93 |
| Day 32 | | | |
| N | 3 | 6 | 9 |
| Mean (SD) | 0.97 (1.19) | 0.29 (0.00) | 0.51 (0.69) |
| Median (Q1, Q3) | 0.29 (0.29, 2.35) | 0.29 (0.29, 0.29) | 0.29 (0.29, 0.29) |
| Min - Max | 0.29 - 2.35 | 0.29 - 0.29 | 0.29 - 2.35 |

Source: Table 14.2.5D, Listing 16.2.6E

**Table 21: Descriptive Statistics of Concentrations of Aspartic Acid [µmoL/L] in Serum during Post-Induction Phase (High Risk Blocks 1 and 2)High Risk Patients - Full Analysis Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **HR block 1** | | | |
| Day 22 | | | |
| N | 7 | 8 | 15 |
| Mean (SD) | 2.00 (2.75) | 1.23 (0.48) | 1.59 (1.87) |
| Median (Q1, Q3) | 1.30 (0.51, 1.51) | 1.47 (0.80, 1.59) | 1.37 (0.51, 1.59) |
| Min - Max | 0.51 - 8.14 | 0.51 - 1.61 | 0.51 - 8.14 |
| Day 25 | | | |
| N | 5 | 7 | 12 |
| Mean (SD) | 4.00 (3.64) | 4.13 (3.05) | 4.08 (3.14) |
| Median (Q1, Q3) | 3.32 (1.66, 3.42) | 3.25 (2.36, 4.32) | 3.29 (2.06, 4.21) |
| Min - Max | 1.30 - 10.28 | 1.75 - 10.71 | 1.30 - 10.71 |
| Day 32 | | | |
| N | 4 | 6 | 10 |
| Mean (SD) | 6.06 (3.78) | 9.94 (5.20) | 8.39 (4.88) |
| Median (Q1, Q3) | 6.45 (3.42, 8.70) | 10.71 (4.25, 13.61) | 7.91 (4.25, 12.79) |
| Min - Max | 1.13 - 10.19 | 3.90 - 16.48 | 1.13 - 16.48 |
| **HR block 2** | | | |
| Day 22 | | | |
| N | 4 | 7 | 11 |
| Mean (SD) | 2.02 (1.20) | 2.01 (1.09) | 2.01 (1.07) |
| Median (Q1, Q3) | 1.50 (1.30, 2.74) | 2.32 (0.51, 2.75) | 2.01 (1.28, 2.75) |
| Min - Max | 1.28 - 3.79 | 0.51 - 3.27 | 0.51 - 3.79 |
| Day 25 | | | |
| N | 2 | 6 | 8 |
| Mean (SD) | 10.26 (8.92) | 6.58 (3.35) | 7.50 (4.72) |
| Median (Q1, Q3) | 10.26 (3.95, 16.56) | 6.38 (3.87, 8.90) | 6.38 (3.91, 9.99) |
| Min - Max | 3.95 - 16.56 | 2.87 - 11.08 | 2.87 - 16.56 |
| Day 32 | | | |
| N | 3 | 6 | 9 |
| Mean (SD) | 7.47 (3.42) | 5.43 (1.96) | 6.11 (2.52) |
| Median (Q1, Q3) | 7.27 (4.15, 10.98) | 4.94 (4.18, 5.13) | 5.13 (4.18, 7.27) |
| Min - Max | 4.15 - 10.98 | 4.05 - 9.32 | 4.05 - 10.98 |

Source: Table 14.2.5E, Listing 16.2.6E

Similar to induction treatment, ASP levels rose during treatment with study drug due to continuous cleavage of ASN.

With regard to evaluation of glutamine concentrations in serum during the post-induction phase, slightly larger decreases were seen in patients administered recombinant asparaginase than patients administered asparaginase medac in HR Blocks 1 and 2). This pattern was also repeated, with respect to increases in glutamic acid serum concentrations.

*Complete Remission Rate and Minimal Residual Disease Status after Induction Phase*

The assessment of CR rate and MRD status was performed after induction treatment, i.e. Day 33 or later if bone marrow was not evaluable on Day 33. Rates of CR and MRD status after induction were comparable in both treatment groups. In each of the treatment groups two patients (2.0%) were not in CR. The MRD status after induction phase was negative in 32 patients (31.7%) in the asparaginase medac group and in 29 patients (29.6%) in the recombinant asparaginase group.

**Table 22: Summary of CR and MRD after Induction Phase - Full Analysis Set (study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **CR** | | | |
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Yes | 97 ( 96.0%) | 90 ( 91.8%) | 187 ( 94.0%) |
| No | 2 ( 2.0%) | 2 ( 2.0%) | 4 ( 2.0%) |
| Not assessable | 0 (0%) | 2 ( 2.0%) | 2 ( 1.0%) |
| NA/ND/NK | 2 ( 2.0%) | 4 ( 4.1%) | 6 ( 3.0%) |
| Difference[a] (rASNase - ASNase medac) | -4.2% | | |
| 95% CI[b] | [-11.90%; 2.81%] | | |
| **MRD status** | | | |
| Number of patients | 101 (100.0%) | 98 (100.0%) | 199 (100.0%) |
| Negative | 32 ( 31.7%) | 29 ( 29.6%) | 61 ( 30.7%) |
| Positive | 60 ( 59.4%) | 63 ( 64.3%) | 123 ( 61.8%) |
| NA/ND/NK | 9 ( 8.9%) | 6 ( 6.1%) | 15 ( 7.5%) |
| Difference[a] (rASNase - ASNase medac) | -2.1% | | |
| 95% CI[b] | [-14.97%; 10.84%] | | |

Source: Listing 16.2.6H
[a] If CR or MRD is not assessable or NA/ND/NK, patient will be considered to be not in CR or MRD positive, respectively.
[b] unconditional exact confidence interval based on Chan and Zhang

*Cumulative incidence of Relapse, Relapse Free Survival and Event-Free Survival*

For the evaluation of cumulative incidence of relapse, RFS and EFS patients were followed up every 12 months ± 2 weeks after randomisation until disease recurrence, death or until the end of the study. Patients who discontinued treatment according to protocol DCOG ALL 10 and patients who received allogenic bone marrow transplantation were not followed up. The median time of follow up was 12 months in both treatment groups.

No relapses occurred during this time in either treatment group. Relapse-free survival was defined as the probability of being alive with no indication of relapse. Since no relapses were documented, RFS was exclusively driven by the four deaths that occurred.

Event-free survival was defined as the time span between date of randomisation and date of death, relapse or premature termination of DCOG ALL 10 protocol - whichever occurred first.

The Kaplan-Meier estimates for RFS and EFS in the asparaginase medac and recombinant asparaginase groups are described below.

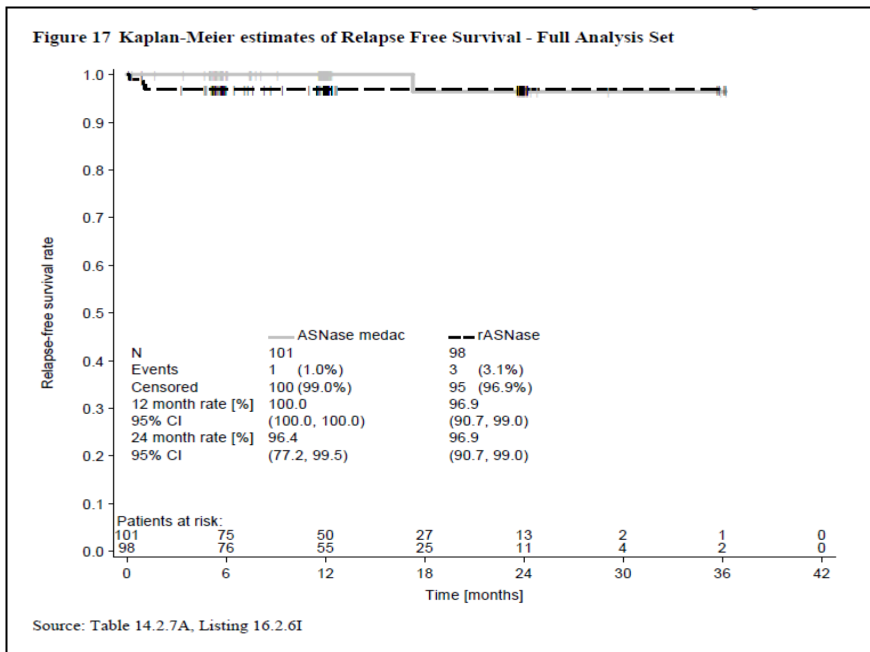



**Figure 8: Kaplan-Meier estimates of Relapse Free Survival - Full Analysis Set (study MC-ASP.5/ALL)**

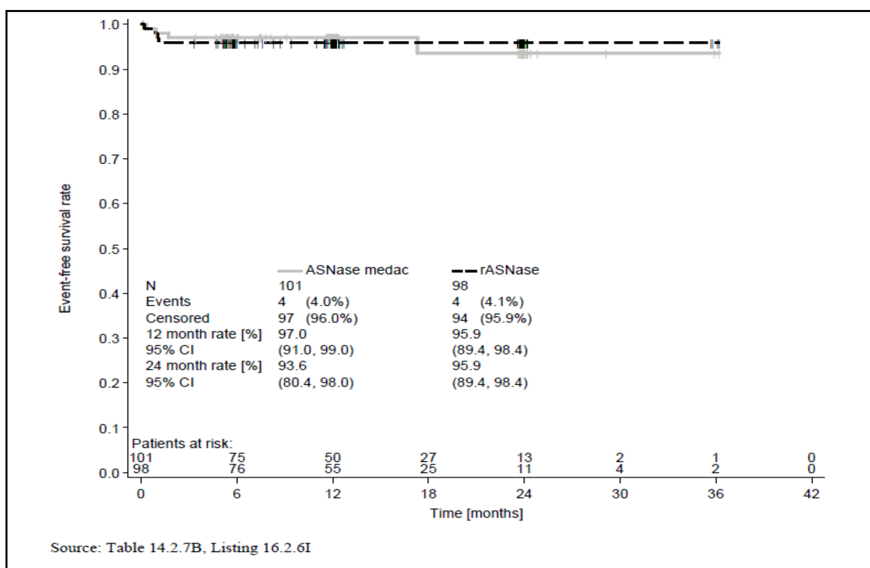



**Figure 9: Kaplan-Meier estimates of Event Free Survival - Full Analysis Set (study MC-ASP.5/ALL)**

*ASN concentrations vs. of ASNase activity*

A scatter diagram was also created showing the ASNase activity levels versus ASN concentrations to assess the relevance of ASNase activity as a biomarker for predicting ASN depletion. Associated Bravais-Pearson and Spearman correlation coefficients were used to judge the strength of the correlations. The fundamental unit of this analysis is the individual blood sample.

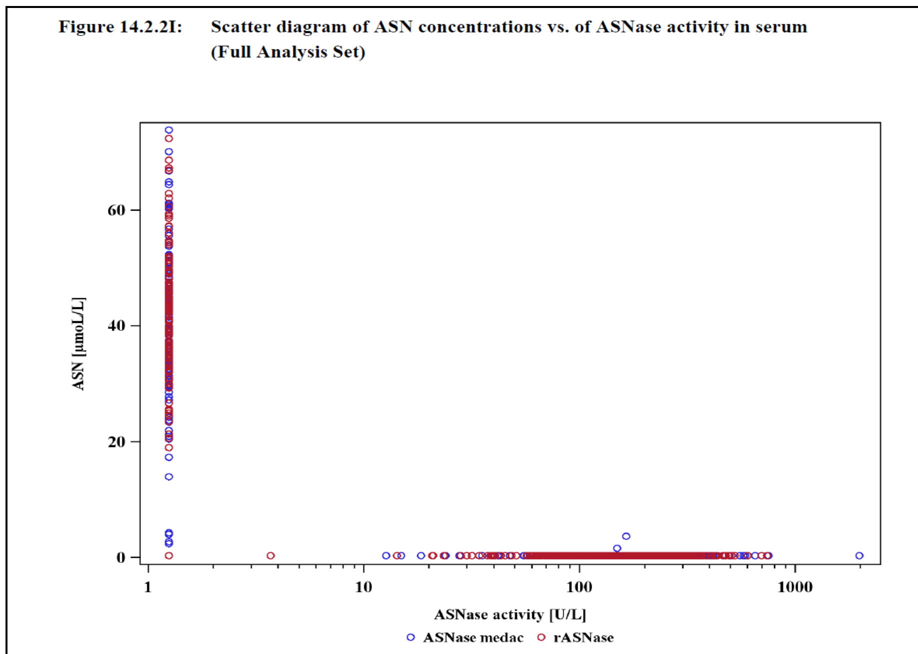



**Figure 10: Scatter diagram of ASN concentrations vs. of ASNase activity in serum (FAS) (study MC-ASP.5/ALL)**

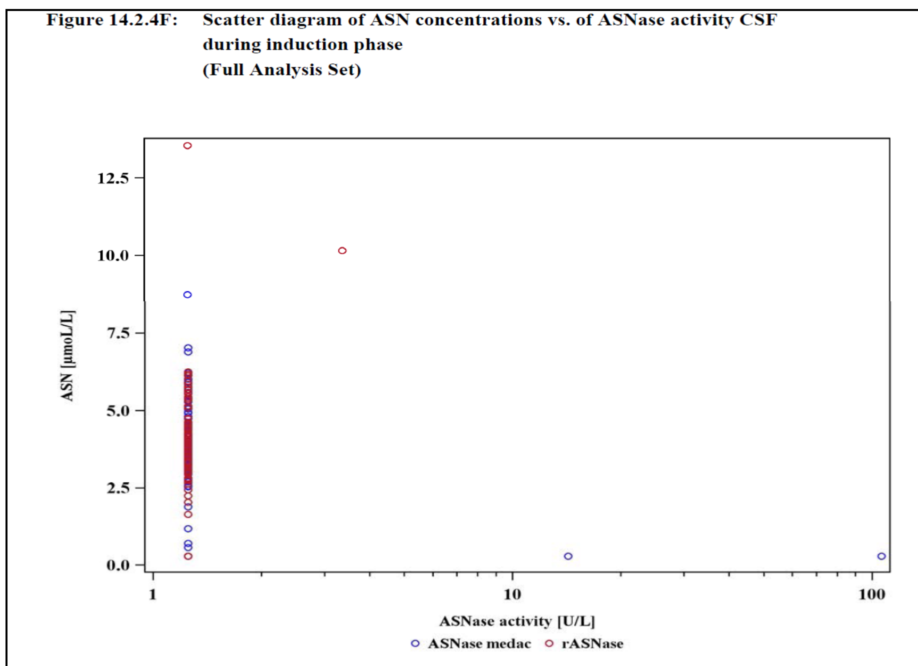



**Figure 11: Scatter diagram of ASN concentrations vs. of ASNase activity in CSF during induction phase (FAS) (study MC-ASP.5/ALL)**

### Ancillary analyses

The following table shows the proportion of patients with ASNase activity in serum < 100 U/L and ≥ 100 U/L during induction phase.

**Table 23: Proportion of patients with ASNase activity in serum < 100 U/L during induction phase by day of protocol (MC-ASP.5/ALL; Evaluation method I – Full analysis set)**

| | ASNase medac | rASNase |
|---|---|---|
| **Day 15** | | |
| Number of patients | 101 (100.0%) | 96 (100.0%) |
| <100 U/L[a] | 19 (18.8%) | 16 (16.7%) |
| ≥ 100 U/L | 67 (66.3%) | 65 (67.7%) |
| Missing | 15 (14.9%) | 15 (15.6%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | -2.3% (0.7104) | |
| Imputation (TRT-MI)[d] | -2.1% (0.6938) | |
| Worst case analysis[e] | -1.4% (0.8378) | |
| **Day 21** | | |
| Number of patients | 101 (100.0%) | 96 (100.0%) |
| <100 U/L[a] | 15 (14.9%) | 16 (16.7%) |
| ≥ 100 U/L | 67 (66.3%) | 67 (69.8%) |
| Missing | 19 (18.8%) | 13 (13.5%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | 1.0% (0.8714) | |
| Imputation (TRT-MI)[d] | 1.8% (0.7266) | |
| Worst case analysis[e] | -3.5% (0.6033) | |
| **Day 27** | | |
| Number of patients | 101 (100.0%) | 96 (100.0%) |
| <100 U/L[a] | 14 (13.9%) | 22 (22.9%) |
| ≥ 100 U/L | 68 (67.3%) | 60 (62.5%) |
| Missing | 19 (18.8%) | 14 (14.6%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | 9.8% (0.1312) | |
| Imputation (TRT-MI)[d] | 9.1% (0.1002) | |
| Worst case analysis[e] | 4.8% (0.4778) | |
| **Day 33** | | |
| Number of patients | 101 (100.0%) | 96 (100.0%) |
| <100 U/L[a] | 11 (10.9%) | 19 (19.8%) |
| ≥ 100 U/L | 71 (70.3%) | 61 (63.5%) |
| Missing | 19 (18.8%) | 16 (16.7%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | 10.3% (0.0904) | |
| Imputation (TRT-MI)[d] | 8.9% (0.0822) | |
| Worst case analysis[e] | 6.8% (0.3135) | |

*Source: Listing 16.2.6A, Section 9.6, Addendum 04 of MC-ASP.5/ALL clinical study report (dated: 10-Aug-2015) and Table Q1-1.1.1, Appendix to this document*
[a] serves as basis for the difference
[b] missing values will be [c]excluded, [d]imputed by treatment-specific geometric mean per day, [e]considered to be <100 U/L
[f] p-value derived by Chi-Square test

**Table 24: Proportion of patients with any ASNase activity in serum <100 U/L during induction phase from day 15 to day 33 of protocol (MC-ASP.5/ALL; Evaluation method I - Full Analysis Set)**

| | ASNase medac | rASNase |
|---|---|---|
| **Day 15 to day 33** | | |
| Number of patients | 101 (100.0%) | 96 (100.0%) |
| <100 U/L in at least one sample[a] | 31 (30.7%) | 34 (35.4%) |
| ≥ 100 U/L in all samples | 60 (59.4%) | 56 (58.3%) |
| No valid sample | 10 (9.9%) | 6 (6.3%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | 3.7% (0.6027) | |
| Imputation (TRT-MI)[d] | 4.7% (0.4809) | |
| Worst case analysis[e] | 1.1% (0.8785) | |

Source: *Listing 16.2.6A, Section 9.6, Addendum 04 of MC-ASP.5/ALL clinical study report (dated: 10-Aug-2015) and Table Q1-1.1.2, Appendix to this document*[a] serves as basis for the difference
[a] serves as basis for the difference
[b] patients with no valid sample will be [c]excluded, [d]imputed by treatment-specific geometric mean per day, [e]considered to be <100 U/L
[f] p-value derived by Chi-Square test

**Table 25: Proportion of <u>samples</u> with ASNase activity in serum <100 U/L during induction phase from day 15 to day 33 of protocol (MC-ASP.5/ALL; Evaluation method I - Full Analysis Set)**

| | ASNase medac | rASNase |
|---|---|---|
| **Day 15 to day 33 (samples)** | | |
| Number of samples | 404 (100.0%) | 384 (100.0%) |
| <100 U/L[a] | 59 ( 14.6%) | 73 ( 19.0%) |
| ≥ 100 U/L | 273 ( 67.6%) | 253 ( 65.9%) |
| Missing | 72 ( 17.8%) | 58 ( 15.1%) |
| Differences[b] (rASNase - ASNase medac) [p-value[f]]: | | |
| Complete case analysis[c] | 4.6% (0.1388) | |
| Imputation (TRT-MI)[d] | 4.4% (0.0978) | |
| Worst case analysis[e] | 1.7% (0.6150) | |

Source: Listing 16.2.6A, Section 9.6, Addendum 04 of MC-ASP.5/ALL clinical study report (dated: 10-Aug-2015) *and Table Q1-1.1.3, Appendix to this document*
[a] serves as basis for the difference
[b] patients with no valid sample will be [c]excluded, [d]imputed by treatment-specific geometric mean per day, [e]considered to be <100 U/L
[f] p-value derived by Chi-Square test

**Summary of main study**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

**Table 26: Summary of efficacy for trial MC-ASP.5/ALL**

| **Title:** Comparative efficacy and safety of two asparaginase preparations in children with previously untreated acute lymphoblastic | | |
|---|---|---|
| Study identifier | **MC-ASP.5/ALL** | |
| Design | Multicentre, randomized, active-controlled, double-blind, parallel-group, phase III | |
| | Duration of treatment: | Induction: 8 infusions<br><br>Post-induction in HR patients:<br>4 infusions in each of HR blocks 1, 2, 4 and 5; 4 infusions in protocol II |
| Hypothesis | Non-inferiority | |
| Treatments groups | Recombinant-asparaginase | DCOG ALL 10 Induction:<br>5000 U/m$^2$ days 12, 15, 18, 21, 24, 27, 30 and 33<br><br>DCOG ALL 10 Post-induction in HR patients:<br>10000 U/m$^2$ days 22, 25, 29 and 32 (HR blocks); days 8, 11, 15 and 18 (protocol II)<br><br>98 randomized patients |
| | asparaginase medac | DCOG ALL 10 Induction:<br>5000 U/m$^2$ days 12, 15, 18, 21, 24, 27, 30 and 33<br><br>DCOG ALL 10 Post-induction in HR patients:<br>10000 U/m$^2$ days 22, 25, 29 and 32 (HR blocks); days 8, 11, 15 and 18 (protocol II)<br><br>101 randomized patients |
| Endpoints and definitions | Primary endpoint | Rate of patients with **complete ASN depletion in serum** during induction treatment.<br>Complete ASN depletion is defined as ASN level below the lower limit of quantification (LLoQ) in each measurement, immediately before asparaginase infusions 2, 4, 6, and 8. |
| | Secondary endpoint | Rate of patients with **complete ASN depletion in CSF**, measured at day 33 and defined as ASN level below the lower limit of quantification (LLoQ). |
| | Secondary endpoint | Trough level of asparaginase activity in blood serum, measured just before the next asparaginase infusion during the induction treatment (infusions 2, 4, 6 and 8). |
| | Secondary endpoint | asparaginase activity levels in CSF, measured during induction treatment at day 33 |
| | Secondary endpoint | Concentration of amino acids ASN, ASP, GLN and GLU in serum (before infusions 2, 4, 6 and 8) and CSF (at day 33). |
| | Secondary endpoint | Complete remission rate at day 33, defined as the presence of <5% leukaemic blasts and regenerating haematopoiesis, without documented extramedullary leukaemia. |
| | Secondary endpoint | MRD status by PCR at day 33: MRD negativity was defined as no MRD detectable with two MRD-PCR targets, which reach sufficient sensitivity: at least one target with a reproducible sensitivity ≤$10^{-4}$ and one target with a reproducible sensitivity ≤$10^{-3}$. |

| | | | |
|---|---|---|---|
| | Secondary endpoint | Relapse rate: relapse is defined as 5% leukaemic blasts in the bone marrow AND/OR leukaemic blasts in the peripheral blood AND/OR leukaemic cells in the CSF. If leukemic cells in CSF are found within $\leq$ 5 WBC/$\mu$l CSF, an extra confirmation is needed by a repeated lumbar puncture (e.g. after 4 weeks) AND/OR leukaemic infiltration elsewhere. | |
| | Secondary endpoint | Event free survival (EFS): relapse, death or premature termination of DCOG-ALL10 are considered events. | |
| End of study | 17 February 2012 | | |
| **Results and Analysis** | | | |
| **Analysis description** | **Primary Analysis** | | |
| Analysis population and time point description | • Full Analysis set: all randomised patients analysed according to the intention-to-treat principle<br>• Per Protocol Set: all randomised patients with at least three evaluable asparagine assessment | | |
| Descriptive statistics and estimate variability | Treatment group | rASNase | asparaginase medac |
| | Number of subject | 98 | 101 |
| | **Primary endpoint** | | |
| | *Complete asparaginase depletion in serum* | | |
| | Yes | 93 (94.9%) | 95 (94.1%) |
| | No | 2 (2.0%) | 2 (2.0%) |
| | Not evaluable | 3 (3.1%) | 4 (4.0%) |
| | Difference (95%CI) (rASNase-ASNase medac)<br>p-value | 0.8% (-6.25%; 8.04%)<br>0.0028 | |
| | **Secondary endpoint** | | |
| | *Complete asparaginase depletion in CSF* | | |
| | Yes | 82 (83.7%) | 88 (87.1%) |
| | No | 1 (1.0%) | 6 (5.9%) |
| | Not evaluable | 15 (15.3%) | 7 (6.9%) |
| | Difference (rASNase-ASNase medac) (95%CI) | -3.5%<br>(-13.67%; 6.58%) | |
| | *Complete remission* | | |
| | Yes | 90 (91.8%) | 97 (96%) |
| | No | 2 (2.0%) | 2 (2.0%) |
| | Not evaluable | 2 (2.0%) | - |
| | Not known | 4 (4.1%) | 2 (2.0%) |
| | Difference (rASNase-ASNase medac) (95%CI) | -4.2%<br>(-11.90%; 2.81%) | |

| | | | |
|---|---|---|---|
| | *Minimal residual disease* | | |
| | Negative | 29 (29.6%) | 32 (31.7%) |
| | Positive | 63 (64.3%) | 60 (59.4%) |
| | Not applicable<br>Not determined<br>Not known | 6 (6.1%) | 9 (8.9%) |
| | Difference<br>(rASNase-ASNase medac)<br>(95%CI) | -2.1%<br>(-14.97%; 10.84%) | |
| | *Relapse Free Survival* | | |
| | At 12 months % (95% CI) | 96.9 (90.7; 99.0) | 100 (100; 100) |
| | At 24 months % (95% CI) | 96.9 (90.7; 99.0) | 96.4 (77.2; 99.5) |
| | *Event Free Survival* | | |
| | At 12 months % (95% CI) | 95.9 (89.4; 98.4) | 97.0 (91.9; 99.0) |
| | At 24 months % (95% CI) | 95.9 (89.4; 98.4) | 93.6 (80.4; 98.0) |
| Notes | | | |

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

Not applicable.

***Clinical studies in special populations***

No studies in special population were submitted.

**Supportive studies**

**STUDY MC-ASP.4/ALL**

This was a single-centre, randomised, double-blind, parallel group phase II clinical trial. The first subject was enrolled on 4 January 2005 and the last subject completed the study on 17 October 2006.

Study objectives

- to compare the pharmacokinetics, pharmacodynamics, efficacy, and safety of recombinant ASNase versus Asparaginase medac during induction treatment in children with de novo ALL.

- to demonstrate that any clinically important difference to the disadvantage of recombinant ASNase is unlikely

Primary endpoint

- Ratio (recombinant L-asparaginase / Asparaginase medac) of the population geometric means of the 72 hours (h) serum concentration (L-asparaginase serum activities) versus time curves ($AUC_{0-72}$) for the first administration of recombinant L-asparaginase and Asparaginase medac.

This was calculated by the log-linear trapezoidal method, i.e., the linear trapezoidal rule is applied up to $t_{max}$ and then the log trapezoidal rule is applied for the remainder of the curve. Analysis of variance

procedures were applied for the confirmatory analysis of the AUC parameter. A 90% confidence interval for the difference in the log-transformed AUCs was calculated using the total between-subject variance. The antilogs of this confidence interval were used to check whether this interval was contained within the predefined equivalence limit of 0.75 and 1.33.

Secondary endpoints

- $C_{max}$, $T_{max}$ (if multiple maxima are observed in the curve, $t_{max}$ is assigned to the first maximum), *λz* (terminal elimination rate constant), $t_{1/2\lambda z}$ (terminal elimination half-life); results from the statistical analysis of $C_{max}$ were not considered for the decision on bioequivalence
- *trough levels of asparaginase activity in serum* during subsequent asparaginase infusions
- *serum and cerebrospinal fluid (CSF) levels of* the following amino acids: *asparagine* (ASN), *aspartic acid* (ASP), *glutamine* (GLN), and *glutamic acid* (GLU)
- *complete remission* (CR) rate on Day 33
- *minimal residual disease* (MRD) *status* on Day 33
- *responder rates with respect to ASN depletion* (at least 95% compared to initial value) in serum and CSF for each day (Day 15 to 33); non-inferiority and superiority were both assessed. 20% of the reference value was considered as non-inferiority margin, also for duration of ASN depletion
- *duration of ASN depletion* response (defined as ASN depletion of at least 95%) in serum and (depletion below the lower limit of quantification) in CSF after the last administration
- *adverse events* between day 12 (first infusion of study drug) until end of phase A of induction treatment (day 35)
- *serum biochemistry, haematology, coagulation screen*

Study design

This study was conducted in a single centre in Rotterdam, Netherlands. A total of 32 patients were randomised to ensure that 30 would qualify for pharmacokinetic and pharmacodynamic analysis.

The study was embedded in the treatment protocol DCOG ALL-10 and conducted within a treatment period of about 4 weeks (see previous trial).

All patients received a combination chemotherapy according to the DCOG ALL-10 protocol including 8 infusions of either recombinant L-asparaginase or Asparaginase medac during phase A of induction treatment. Starting with the first L-asparaginase infusion on day 12, the patients received study medication every third day, ending with the eighth infusion on Day 33.

This study required the following dose of L-asparaginase during induction treatment to be administered: 5,000 U/m²; in case of appearance of any allergic reaction, treatment with asparaginase preparations had to be stopped. Within the trial, the study medication (recombinant L-asparaginase or Asparaginase medac) was only used during induction treatment. Patients were treated post-induction with pegaspargase.

Study population

*Main inclusion criteria*

- Previously untreated ALL
- Morphological proof of ALL and diagnosis made from bone marrow morphology with ≥ 25% blasts
- Age ≥ 1 year and ≤ 18 years

*Main Exclusion criteria*

- General health status according to Karnofsky / Lansky score < 40%
- Pre-existing coagulopathy (e.g. haemophilia)
- Patients with known genetic prothrombotic risk factors
- Pre-existing pancreatitis, kidney or liver insufficiency
- Other current malignancies

Statistical plan

The confirmative objective of this trial is to show that the 90% confidence interval of the ratio of the population geometric means of the 72 h asparaginase serum concentration versus time curves ($AUC_{0\text{-}72h}$) for the first administration of recombinant asparaginase (test product) and Asparaginase medac (reference product) is within the equivalence limit 0.75 to 1.33. This wider acceptance range was chosen due to the following reasons: substantial variance of the relative bioavailability (parallel group design, paediatric patients); wide therapeutic range of asparaginase treatment; no safety and/or efficacy concerns; paediatric patient population (age ≥1 year up to ≤18 years).

*Analysis populations*

- ITT population - Patients randomised into the study who had received at least one asparaginase infusion. This was used for the safety analysis.
- PK population - All patients who had been administered the first dose of the study medication and had completed the study up to Day 15 according to the study protocol without serious deviations (i.e. at least 50% missing samples per asparaginase concentration profile in the 0-72 h interval, significant deviation from scheduled time).
- Per protocol population - All patients who had been administered the first seven doses of the study medication and had completed the study up to Day 33 before administration of the eighth dose without serious deviations which might have influenced the ASN concentrations in serum and CSF.

***Results***

Disposition of patients

Altogether 32 children with previously untreated acute lymphoblastic leukaemia participated in the study. One patient discontinued due to a SAE (viral hepatitis) after 7 of 8 scheduled infusions. All other patients completed the entire course of treatment. All enrolled patients were included in the ITT and PP populations. However, 2 patients were excluded from the PK population.

Demographic and baseline characteristics

**Table 27: Demographic data (ITT population) (Study MC-ASP.4/ALL)**

| Characteristic | Recombinant ASNase (test) | Asparaginase medac™ (reference) |
|---|---|---|
| Age [years]<br>Median, range | 4.5<br>2 / 14 | 4.5<br>1/11 |
| Height [cm]<br>Median, range | 114.0<br>89/167 | 106.5<br>84 /152 |
| Body weight [kg]<br>Median, range | 19.55<br>11.8/76.0 | 17.40<br>10.0/40.8 |
| Body surface area [$m^2$]<br>Median, range | 0.790<br>0.54/1.88 | 0.725<br>0.48/1.22 |
| Sex<br>male / female | 9 / 7 | 8 / 8 |

Source: Table 14.1.1

**Table 28: Disease status (ITT population) (Study MC-ASP.4/ALL)**

| Category/parameter | Recombinant ASNase (test) | Asparaginase medac™ (reference) |
|---|---|---|
| Peripheral blood/ WBC [x10$^9$/L] Median, range | 9.800<br>0.73 / 578.00 | 4.000<br>0.60 / 109.00 |
| Peripheral blood/ Peripheral blasts [%] Median, range | 50.5<br>0 / 96 | 28.0<br>0 / 79 |
| Bone marrow aspirate/ Marrow blasts [%] Median, range | 92.8<br>59.2 / 97.0 | 90.1<br>33.8 / 98.6 |
| Immunophenotype (number of patients) | Pre-pre-B-ALL: 1<br>Common ALL: 8<br>Pre-B-ALL: 4<br>T-ALL: 3 | Pre-pre-B-ALL: 1<br>Common ALL: 10<br>Pre-B-ALL: 3<br>T-ALL: 2 |
| Genetics (number of patients) | TEL/AML 1: 2<br>TEL/AML 1, other: 3<br>Other: 9<br>No aberrations: 2 | BCR/ABL: 1<br>TEL/AML 1: 5<br>MLL-AF4, other: 1<br>Other: 9 |
| Bone marrow aspirate/ Cellularity status (number of patients) | Hypocellular: 3<br>Hypercellular: 11<br>Packed (intensely hypercellular): 2 | Hypocellular: 2<br>Hypercellular: 14 |

Source: Table 14.1.2

WBC counts in peripheral blood ranged from 0.6 to 227×10$^9$ WBC/L, and one patient had an extreme value of 578 ×10$^9$ WBC/L, with up to 96% peripheral blasts.

Pharmacokinetic results

All subjects receiving test or reference treatment exhibited measurable asparaginase serum activities. Immediately after infusion, geometric mean asparaginase serum activity was 3231.19 U/L following recombinant asparaginase infusion and 3303.04 U/L following Asparaginase medac treatment. After 72h, the geometric mean asparaginase serum activity was 123.18 U/L following recombinant asparaginase infusion and 174.96 U/L following Asparaginase medac treatment. Geometric mean serum activity vs. time profiles up to 72 h following administration of the first eight doses is shown below (lin-lin scale).

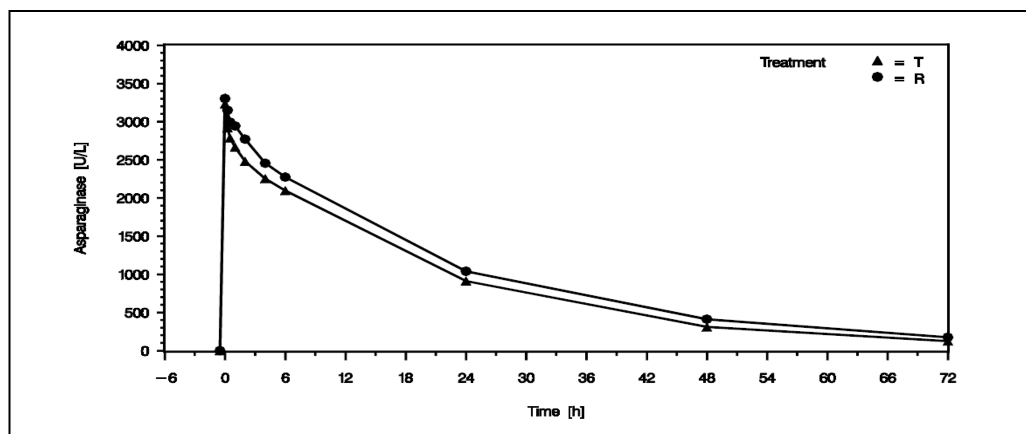



**Figure 12: Serum asparaginase activity vs. time [h] following the first of the eight infusions - Geometric means (PK population) (Study MC-ASP.4/ALL)**

The geometric mean of $AUC_{0-72h}$ was 58356.9 U*h/L following recombinant asparaginase infusion and 67846.4 U*h/L following Asparaginase medac treatment. For $t_{max}$, medians of both treatments were zero indicating that $C_{max}$ was reached immediately after infusion for most patients.

**Table 29: Pharmacokinetic parameters of serum activities of asparaginase (PK population) (Study MC-ASP.4/ALL)**

| Parameter | | Recombinant ASNase (MC1003, test) N=14 | Asparaginase medac™ (MC0904, reference) N=16 |
|---|---|---|---|
| $AUC_{0\text{-}72h}$ [U*h/L] | geom. Mean (SD)<br>Median<br>Min/Max | 58356.9 (1.20)<br>60164.5<br>38626.8/80764.3 | 67846.4 (1.16)<br>69135.6<br>49243.8/83850.1 |
| $C_{max}$ [U/L] | geom. Mean (SD)<br>Median<br>Min/Max | 3350.4 (1.22)<br>3526.7<br>2231.3 /4525.5 | 3645.5 (1.18)<br>3699.8<br>2898.2/4968.0 |
| $t_{max}$ [h] | geom. Mean (SD)<br>Median<br>Min/Max | 0.3162 (3.83)<br>0<br>0/2.0000 | 0.2409 (2.32)<br>0<br>0/0.5000 |
| $\lambda_z$ [h] | geom. Mean (SD)<br>Median<br>Min/Max | 0.0405 (1.18)<br>0.0400<br>0.0302/0.0553 | 0.0369 (1.24)<br>0.0374<br>0.0253/0.0544 |
| $t_{1/2\ \lambda z}$ [h] | geom. Mean (SD)<br>Median<br>Min/Max | 17.1153 (1.18)<br>17.3295<br>12.5392/22.9148 | 18.8055 (1.24)<br>18.5499<br>12.7322/27.3761 |

Source: Table 14.2.1.2

Analysis of variance (ANOVA) and 90% confidence intervals (CI) of log-transformed pharmacokinetic parameters were calculated for serum activities of asparaginase. The point estimate of $AUC_{0\text{-}72h}$ for the treatment ratio test/reference was 86.01 (90% CI: 77.52-95.43), for $C_{max}$ 91.91 (90% CI: 82.08-102.91), for $t_{1/2\lambda z}$ 91.01 (90% CI: 80.78-102.54), and for λz 109.88 (90% CI: 97.53-123.79). For $AUC_{0\text{-}72h}$, the treatment comparison showed that the 90% CI was contained within the widened acceptance range for equivalence of 75% to 133%. Nevertheless a significant difference between the two treatments (p=0.020) was observed. No significant difference between the two treatments was obtained for $C_{max}$ (p=0.215), t1/2λz and its equivalent λz (p=0.190 for both).

**Table 30: Descriptive of serum trough activities of asparaginase (PK population) (Study MC-ASP.4/ALL)**

| Day | | Recombinant ASNase (MC1003, test) N=14 | Asparaginase medac™ (MC0904, reference) N=16 |
|---|---|---|---|
| 15 | geom. Mean (SD)<br>Median<br>Min/Max | 123.18 (1.888)<br>137.80<br>37.5/279.6 | 174.96 (1.733)<br>195.55<br>37.5/345.8 |
| 18 | geom. Mean (SD)<br>Median<br>Min/Max | 162.48 (1.480)<br>164.75<br>84.9/282.2 | 220.09 (1.577)<br>204.20<br>115.8/488.5 |
| 21 | geom. Mean (SD)<br>Median<br>Min/Max | 121.60 (1.666)<br>121.60<br>37.5/275.8 | 188.69 (1.664)<br>163.90<br>81.8/496.0 |
| 24 | geom. Mean (SD)<br>Median<br>Min/Max | 157.78 (1.423)<br>150.05<br>102.7/369.4 | 183.31 (1.985)<br>207.60<br>37.5/398.1 |
| 27 | geom. Mean (SD)<br>Median<br>Min/Max | 139.34 (1.552)<br>145.20<br>76.7/291.3 | 202.23 (1.900)<br>189.30<br>37.5/507.3 |
| 30 | geom. Mean (SD)<br>Median<br>Min/Max | 160.43 (1.840)<br>162.20<br>37.5/324.8 | 205.07 (1.645)<br>193.60<br>106.3/499.5 |
| 33 | geom. Mean (SD)<br>Median<br>Min/Max | 145.89 (2.050)<br>141.45<br>37.5/394.5 | 174.92 (1.771)<br>179.25<br>37.5/423.8 |
| 39 | geom. Mean (SD)<br>Median<br>Min/Max | 20.14 (4.422)<br>21.10<br>1.3/177.0 | 22.46 (2.447)<br>16.30<br>9.5/161.8 |

Source: Table 14.2.1.1

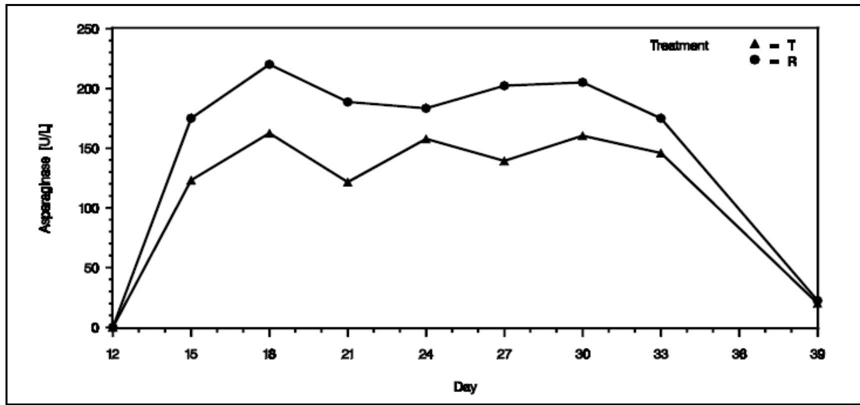



**Figure 13: Serum asparaginase activity [U/L], pre-dose and subsequent days Geometric means (PK population) (Study MC-ASP.4/ALL)**

Most individual asparaginase activities after Day 39 (i.e., Days 45, 52, 59, and 64), were below the lower limit of quantitation (LLoQ) and therefore means were not calculated and displayed. The observed average trough activities under the recombinant L-asparaginase treatment were lower than those observed under the Asparaginase medac treatment. However, trough levels were above the desired threshold of >100 U/L in both groups.

Pharmacodynamic results

*Asparagine concentrations*

Mean asparagine serum concentrations dropped after the first infusion from the pre-dose concentrations of 41.83 µmoL/L (test) and 42.52 µmoL/L (reference) to below LLoQ (BLLQ) of 0.5 µmoL/L of the bioanalytical method under both treatments until Day 33, thereafter, asparagine concentrations in serum increased again.

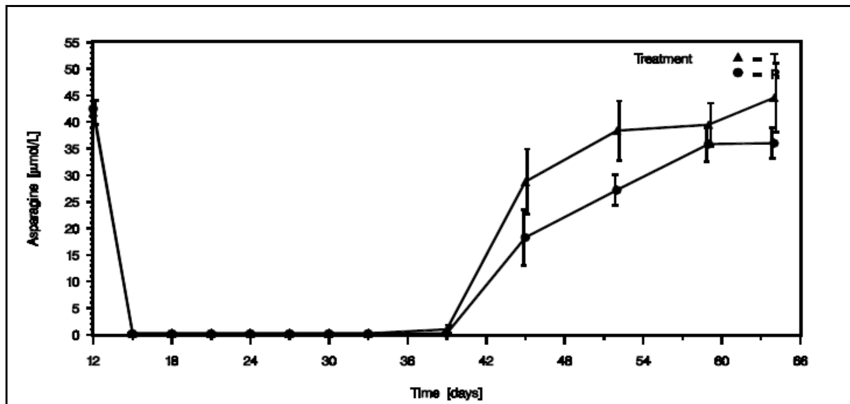



**Figure 14: Asparagine serum concentrations [µmoL/L]. Arithmetic means and standard errors (Study MC-ASP.4/ALL)**

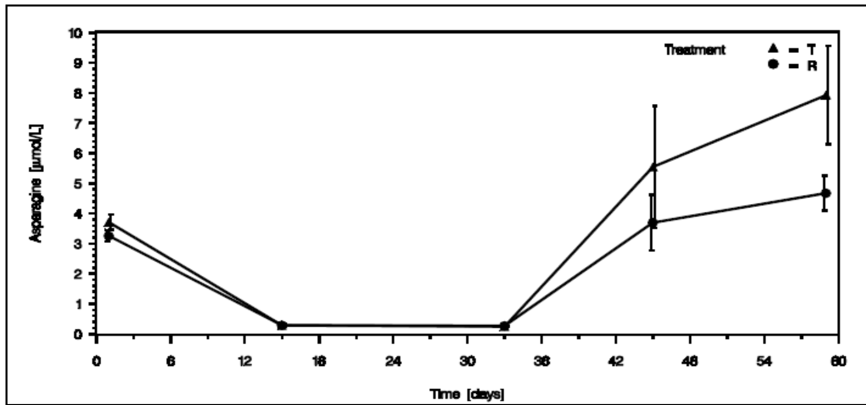



**Figure 15: Asparagine concentration in CSF [µmol/L]. Arithmetic means and standard errors (Study MC-ASP.4/ALL)**

In CSF, the mean asparagine concentrations dropped from 3.71 µmoL/L (test) and 3.26 µmoL/L (reference) to <LLoQ under both treatments on Day 15 and Day 33. The mean asparagine depletion relative to baseline was 91.95% (test) and 90.99% (reference) on Day 15 and 92.92% and 91.28%, respectively, on Day 33.

*Aspartic acid concentrations*

During both asparaginase treatments, aspartic acid concentrations in serum increased.

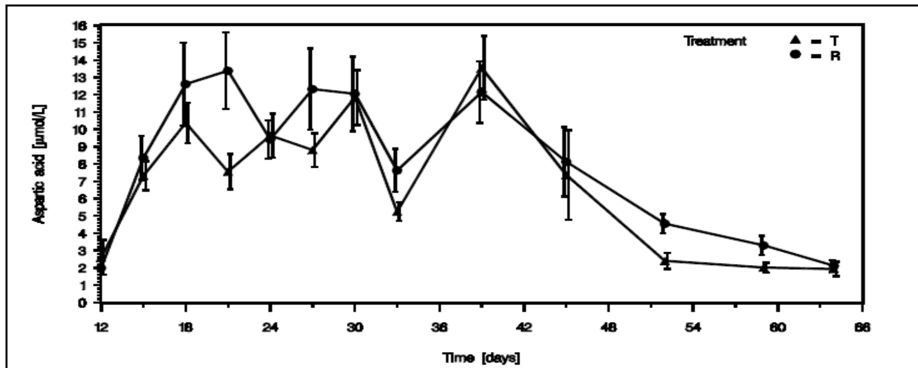



**Figure 16: Aspartic acid [µmoL/L] serum concentrations. Arithmetic means (SE) (Study MC-ASP.4/ALL)**

In CSF, only three out of 32 patients had measurable CSF concentrations for aspartic acid. These patients were under reference treatment.

*Glutamine concentrations*

Arithmetic mean glutamine serum concentrations decreased under both treatments on Day 15 and Day 18; from Day 21 up to day 33 concentrations increased following the recombinant L-asparaginase treatment and decreased following the Asparaginase medac treatment.

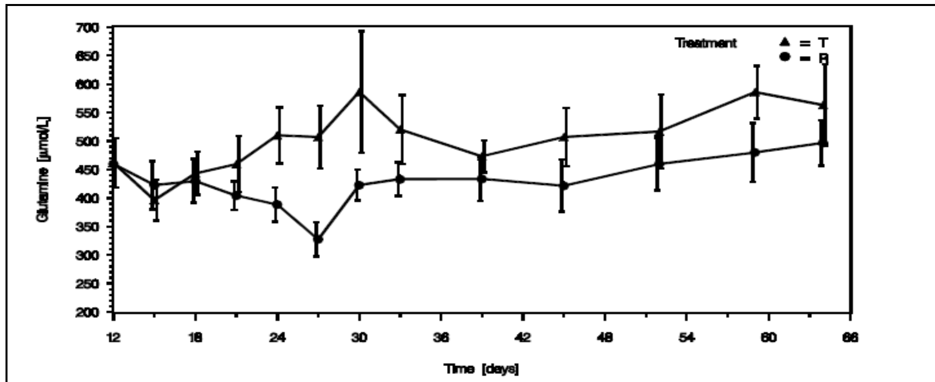



**Figure 17: Glutamine serum concentrations [µmoL/L]. Arithmetic means (SE) (Study MC-ASP.4/ALL)**

Mean glutamine CSF concentration showed slight increases following the recombinant L-asparaginase treatment and rather decreased following the Asparaginase medac treatment.

*Glutamic acid concentrations*

Arithmetic mean glutamic acid serum concentrations increased after first infusion from pre-dose concentration of 38.94 µmoL/L (test) and 47.99 µmoL/L (reference) to 153.19 µmoL/L and 170.95 µmoL/L respectively. Thereafter, glutamic acid concentrations in serum decreased under both treatments.

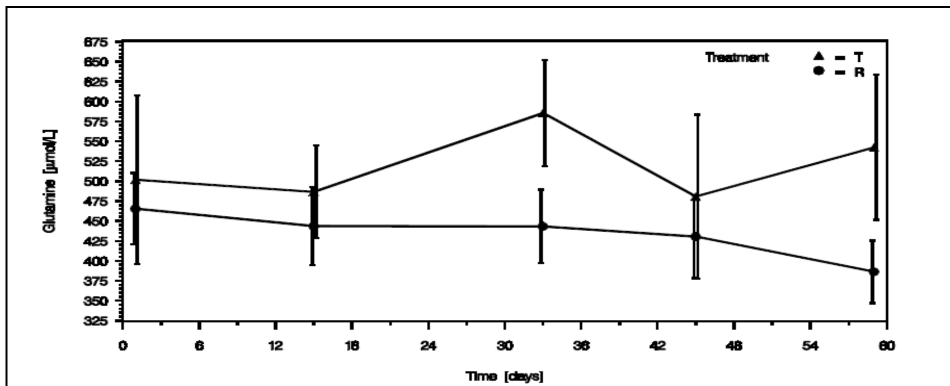



**Figure 18: Glutamic acid serum concentration [µmoL/L]. Arithmetic means (SE) (Study MC-ASP.4/ALL)**

Mean glutamic acid CSF concentrations increased under both treatments versus baseline and remained increased after treatment under recombinant L-asparaginase but returned to baseline under the asparaginase medac.

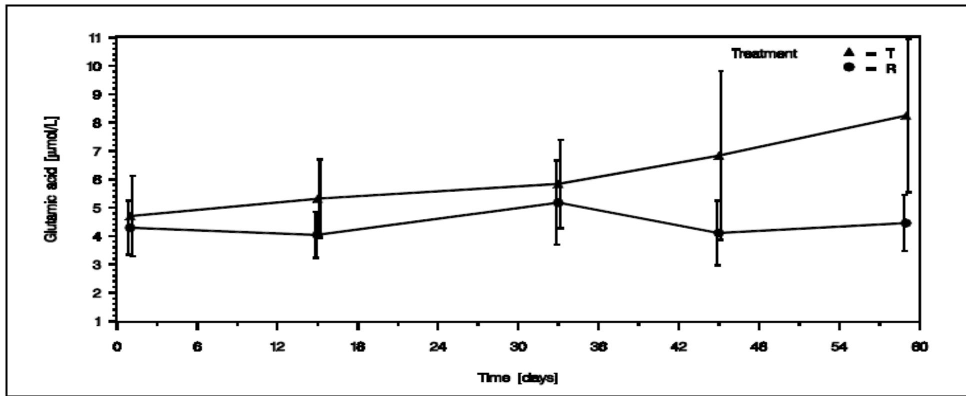



**Figure 19: Glutamic acid [µmoL/L] CSF concentrations (Study MC-ASP.4/ALL)**

*Duration of ASN depletion in serum*

The mean ± SD duration of ASN depletion in serum after last dose of ASNase was 7.58 ± 3.15 days under the rASNase treatment (n = 12) and 9.00 ± 3.46 days under the ASNase medac treatment (n = 11), the log-rank test did not show a significant difference (p = 0.2111). Respective medians for this parameter were 6.5 days versus 7 days. Individual ASN recovery curves differed substantially in both groups.

Looking on the number of patients with complete ASN depletion after ASNase treatment shows that all patients with samples at day 39 (6 days after last ASNase dose) except one in the rASNase group were still completely depleted whereas at day 45 only 3 and 4 patients were still completely depleted in the rASNase and ASNase medac group, respectively. All patients in both groups had reached normal ASN values at day 52.

The number and proportion of patients with ASNase activities > 100 U/L after end of induction treatment.

**Table 31: Proportion of patients with ASNase activity >100 U/L**

| Day | rASNase | | ASNase medac | |
|---|---|---|---|---|
| | No. of evaluable patients | ASNase activity > 100 U/L [n (%)] | No. of evaluable patients | ASNase activity > 100 U/L [n (%)] |
| 33 | 16 | 14 (87.5%) | 16 | 15 (93.8%) |
| 39 | 12 | 2 (16.7%) | 10 | 1 (10%) |
| *Data source: CSR table 16.2.5.1.1* | | | | |

No patient in both groups had a measurable ASNase activity level at day 45 and beyond. Data on duration of ASNase activity during study drug administration period, data on proportion of samples <100 U/L and summary data on time to recovery of pre-study ASN levels are presented below.

Duration of ASNase activity in serum ≥ 100 U/L was comparable in both groups.

**Table 32: Duration of ASNase activity in serum ≥ 100 U/L during induction phase from day 12 (immediately after infusion) to day 64 (MC-ASP.4/ALL - ITT / per-protocol population)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Day 12 (0 h) to day 64** | | | |
| Number of patients | 16 (100.0%) | 16 (100.0%) | 32 (100.0%) |
| At least one post-baseline sample >= 100 U/L | 16 (100.0%) | 16 (100.0%) | 32 (100.0%) |
| Duration of values >=100 U/L [days][a]: | | | |
| N | 16 | 16 | 32 |
| Mean (SD) | 22.9 (5.2) | 21.2 (3.6) | 22.0 (4.5) |
| Median (Q1, Q3) | 22.0 (22.0, 22.0) | 22.0 (22.0, 22.0) | 22.0 (22.0, 22.0) |
| Min, Max | 10, 36 | 13, 26 | 10, 36 |
| p-value[b] | | 0.3296 | |

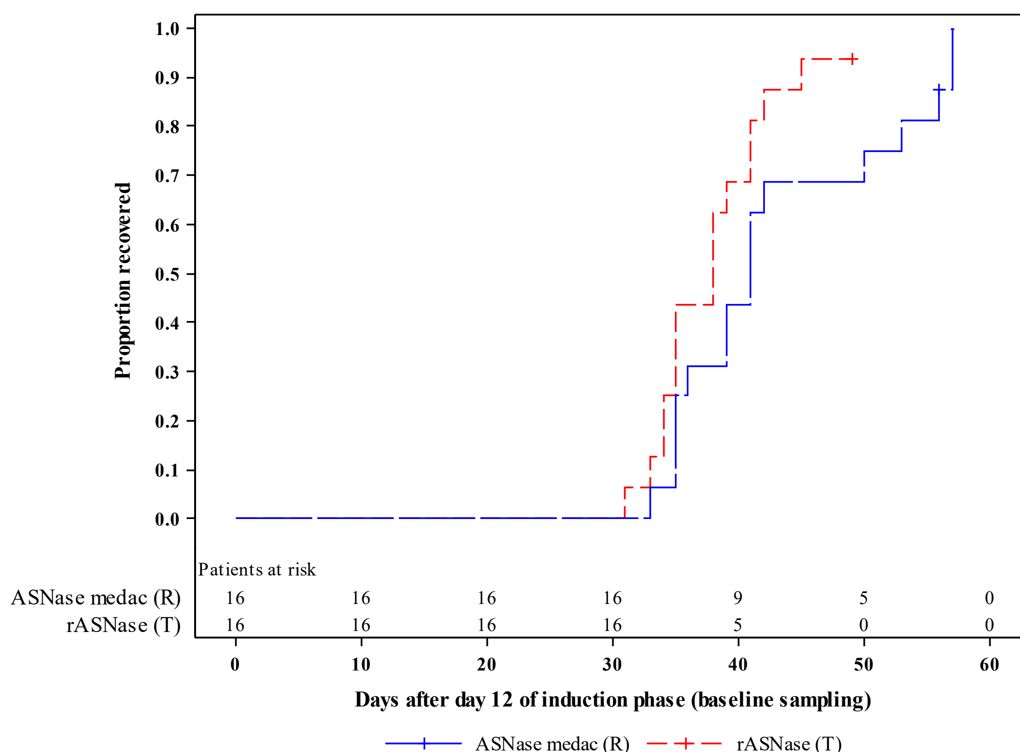
Source: Listing Q14-1, CSR Table 16.2.5.1.1
[a] day of last observation >=100 U/L minus day of first observation >=100 U/L plus 1 (based on samp ling dates)
[b] two-sided p-value derived by Wilcoxon-Mann-Whitney test

**Table 33: Proportion of samples with ASNase activity in serum <100 U/L during induction phase from day 12 (immediately after infusion) to day 64 (MC-ASP.4/ALL - ITT / per-protocol population)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Day 12 (0 h) to day 64 (samples)** | | | |
| Number of samples | 336 (100.0%) | 336 (100.0%) | 672 (100.0%) |
| <100 U/L[a] | 66 ( 19.6%) | 87 ( 25.9%) | 153 ( 22.8%) |
| ≥100 U/L | 232 ( 69.0%) | 214 ( 63.7%) | 446 ( 66.4%) |
| Missing | 38 ( 11.3%) | 35 ( 10.4%) | 73 ( 10.9%) |
| Differences[b] (rASNase - ASNase medac) (p-value[f]): | | | |
| Complete case analysis[c] | | 6.8% (0.0580) | |
| Imputation (TRT-MI)[d] | | 4.2% (0.2274) | |
| Worst case analysis[e] | | 5.4% (0.1416) | |

Source: CSR Table 16.2.5.1.1
[a] serves as basis for the difference
[b] missing values will be [c]excluded, [d]imputed by treatment-specific geometric mean per time point, [e]considered to be <100 U/L
[f] p-value derived by Chi-Square test

Time to recovery was defined as time to reach ASN values within reference range. In particular, based on MC-ASP.5/ALL (used because of the larger sample size; n = 197), recovery of ASN was defined as having reached the lower bound of the asymptotic 95% prediction interval, calculated as mean minus 2 x SD, i.e. 44.04 - 2 x 10.74 = 22.56 µM.



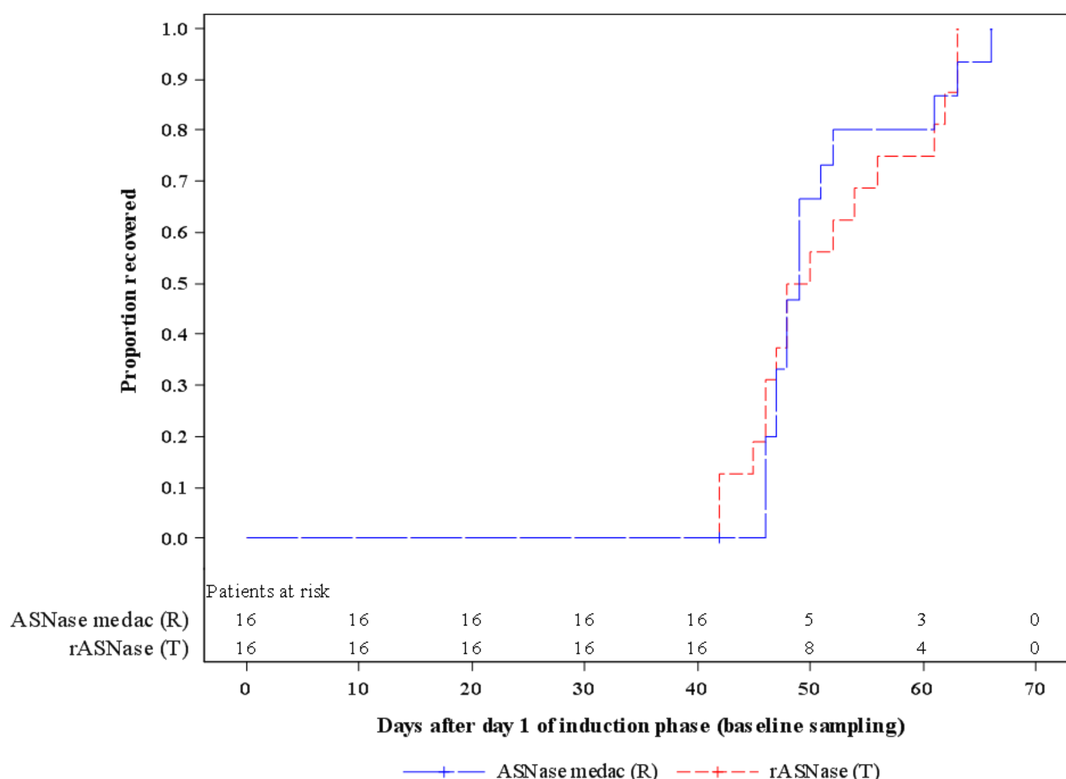

Source: CSR Table 16.2.6.1.1

**Figure 20: Time to recovery of ASN in serum (≥ 22.56 µM in any post-baseline sample) during induction phase from day 12 to day 64 (MC-ASP.4/ALL - Intent-to-treat / per-protocol population)**

**Table 34: Time to recovery (days) of ASN in serum (≥ 22.56 µM in any post-baseline sample) during induction phase from day 12 to day 64 (MC-ASP.4/ALL - ITT / per-protocol population)**

| | ASNase medac | rASNase |
|---|---|---|
| Number of patients | 16 | 16 |
| Recovered | 15 (94%) | 15 (94%) |
| Not recovered | 1 (6%) | 1 (6%) |
| Median (Q1, Q3) | 41.0 (35.5, 51.5) | 38.0 (34.5, 41.0) |
| Minimum, Maximum | 33, 57 | 31, 45 |
| p-value (Log-Rank test) | 0.0605 | |
| p-value (Wilcoxon) | 0.0856 | |
| *Note: Time is calculated as the number of days after day 12 of induction phase (baseline sampling)* | | |

The respective values for CSF are: mean (SD): 4.06 (1.28); median (Q1/Q3): 3.99 (3.34, 4.51); range: 0.29 - 13.54. Recovery of ASN was defined as having reached the lower bound of the asymptotic 95% prediction interval, i.e. mean minus 2 x SD = 4.06 – 2 x 1.28) : 1.5 µM.




Source: CSR Table 16.2.6.2.1

**Figure 21: Time to recovery (days) of ASN in CSF (≥ 1.5 µM in any post-baseline sample) during induction phase from day 1 to day 59 (MC-ASP.4/ALL - ITT / per-protocol population)**

**Table 35: Time to recovery (days) of ASN in CSF (≥ 1.5 µM in any post-baseline sample) during induction phase from day 1 to day 59 (MC-ASP.4/ALL - ITT / per-protocol population)**

| | ASNase medac | rASNase |
|---|---|---|
| Number of patients | 16 | 16 |
| Recovered | 15 (94%) | 16 (100%) |
| Not recovered | 1 (6%) | 0 (0%) |
| Median (Q1, Q3) | 49.0 (47.0, 52.0) | 49.0 (46.0, 58.5) |
| Minimum, Maximum | 46, 66 | 42, 63 |
| p-value (Log-Rank test) | 0.9185 | |
| p-value (Wilcoxon) | 0.8750 | |
| *Note: Time is calculated as the number of days after day 1 of induction phase (baseline sampling)* | | |

CR rate, MRD rate and responder rates

Efficacy of the treatments was determined by evaluating the CR rate and MRD rate, responder rates of the patients with respect to ASN depletion and duration of the depletion response.

There were less than 5% leukaemic blasts in bone marrow in both treatment groups. The mean percentage of leukaemic blasts in bone marrow was 1.18% for the patients receiving recombinant L-asparaginase and 0.55% for the patients receiving Asparaginase medac. No leukaemic blasts were observed in peripheral blood. In CSF, in 1 of 16 patients receiving recombinant L-asparaginase treatment, leukaemic blasts were observed, and in 1 of the 16 patients receiving Asparaginase medac treatment, the status was not determined. None of the patients in either group showed other extramedullary leukaemia manifestations, and all had regained haematopoiesis. Except for one patient, all patients showed complete remission.

**Table 36: Efficacy assessment – descriptive statistics – ITT population / PP population (Study MC-ASP.4/ALL)**

Table 14.2.4.1 (page 1 of 2): 17JUL2007
Efficacy assessment. Descriptive statistics.

Intent-to-treat (ITT) population / per-protocol (PP) population

| Category | Parameter | Treat-ment | Sex | N | Mean | SD | CV | Minimum | Median | Maximum |
|---|---|---|---|---|---|---|---|---|---|---|
| Bone marrow | Leukaemic blasts [%] | T | male | 9 | 0.80 | 0.618 | 77.31 | 0.0 | 0.60 | 1.9 |
| | | | female | 7 | 1.66 | 1.204 | 72.65 | 0.4 | 1.00 | 3.4 |
| | | | TOTAL | 16 | 1.18 | 0.988 | 84.11 | 0.0 | 0.90 | 3.4 |
| | | R | male | 8 | 0.88 | 1.079 | 123.35 | 0.0 | 0.40 | 2.8 |
| | | | female | 8 | 0.23 | 0.377 | 167.56 | 0.0 | 0.00 | 1.0 |
| | | | TOTAL | 16 | 0.55 | 0.850 | 154.56 | 0.0 | 0.30 | 2.8 |

All patients responded to the treatment on Day 15 to 33, showing an at least 95% depletion of ASN compared to the initial value in serum for the respective treatment day, although a small number of samples in both treatment arms (in both serum and CSF analyses) were missing and could not be included in the analysis.

**Table 37: Descriptive statistics of responder status defined by ASN (serum depletion at least 95% of initial value, CSF: BLLQ) – ITT population/PP population (Study MC-ASP.4/ALL)**

Table 14.2.4.2 (page 3 of 4): 17JUL2007
Descriptive statistics of Responder status defined by ASN (serum: depletion at least 95% of initial value, CSF: BLLQ).

Intent-to-treat (ITT) population / per-protocol (PP) population

| Matrix | Parameter | Treat-ment | | N | % |
|---|---|---|---|---|---|
| Serum | Responder (ASN depletion) on day 15 to 33 | T | Yes | 15 | 100.0 |
| | | | ms | 1 | |
| | | R | Yes | 10 | 100.0 |
| | | | ms | 6 | |
| | Duration (days) of ASN depletion after the last administration | T | ms | 3 | |
| | | | 0 | 1 | 7.7 |
| | | | 1-9 | 9 | 69.2 |
| | | | >9 | 3 | 23.1 |
| | | R | ms | 4 | |
| | | | 0 | 1 | 8.3 |
| | | | 1-9 | 7 | 58.3 |
| | | | >9 | 4 | 33.3 |

Table 14.2.4.2 (page 4 of 4): 17JUL2007
Descriptive statistics of Responder status defined by ASN (serum: depletion at least 95% of initial value, CSF: BLLQ).

Intent-to-treat (ITT) population / per-protocol (PP) population

| Matrix | Parameter | Treat-ment | | N | % |
|---|---|---|---|---|---|
| CSF | Responder (ASN depletion) on day 15 | T | Yes | 15 | 93.8 |
| | | | No | 1 | 6.3 |
| | | R | Yes | 13 | 86.7 |
| | | | ms | 1 | |
| | | | No | 2 | 13.3 |
| | Responder (ASN depletion) on day 33 | T | Yes | 16 | 100.0 |
| | | R | Yes | 15 | 93.8 |
| | | | No | 1 | 6.3 |
| | Responder (ASN depletion) on day 15 to 33 | T | Yes | 15 | 93.8 |
| | | | No | 1 | 6.3 |
| | | R | Yes | 13 | 86.7 |
| | | | ms | 1 | |
| | | | No | 2 | 13.3 |

For duration of ASN depletion in serum after the last asparaginase administration, 3 patients under recombinant L-asparaginase and 4 under Asparaginase medac treatment had a depletion with a duration

of more than 9 days. 3 patients treated with recombinant L-asparaginase and 4 treated with Asparaginase medac had missing samples on Day 39 so duration of the depletion could not be determined.

**Table 38: Descriptive statistics and log-rank test for duration (days) of ASN depletion in serum after the last administration – ITT population / PP population (Study MC-ASP.4/ALL)**

Table 14.2.4.3: 17JUL2007
Descriptive statistics and log-rank test for duration (days) of ASN depletion in serum after the last administration.

Intent-to-treat (ITT) population / per-protocol (PP) population

| Treat-ment | N | Mean | SD | CV | Minimum | Median | Maximum | Censored | P-value Log-rank |
|---|---|---|---|---|---|---|---|---|---|
| T | 12 | 7.58 | 3.15 | 41.49 | 4 | 6.5 | 15 | 0 | 0.2111 |
| R | 11 | 9.00 | 3.46 | 38.49 | 6 | 7 | 15 | 0 | |

The exploratory comparisons did not show significant differences between the two treatments for the responder rates of patients with respect to ASN depletion in serum (at least 95% compared to initial value) and CSF (percentage of patients with ASN < LLoQ) for each day (Days 15 to 33).

Correlation of Asparagine in serum with asparaginase activity

The asparagine concentrations in serum correlated significantly (Spearman r = -0.7084 for test and r = -0.6813 for reference; p<0.0001) to asparaginase serum concentrations under both treatments, that is, the higher the concentrations of asparaginase, the lower the asparagine concentrations.

The mixed model analysis of asparagine depletion in serum from Day 15 to Day 33 showed least square (LS) means for the recombinant asparaginase treatment of 99.38 (μT) and of 99.40 (μR) for the Asparaginase medac treatment. The lower limits of the two-sided 95% confidence interval for the treatment difference (μT- μR) derived from the mixed model is -0.0243 (absolute) and -0.0244 (expressed in % of the LS mean of the reference). Non-inferiority of recombinant asparaginase (Test) in comparison to Asparaginase medac (Reference) with respect to ASN depletion in serum from Day 15 to 33 was concluded as the lower limit of the two-sided 95% confidence interval for the treatment difference (μT-μR) derived from the mixed model is above the corresponding Δ (−0.2 μR).

ANOVA of ASN depletion in CSF was not done because nearly all values on Days 15 and 33 were below the LLoQ.

Asparaginase treatment significantly correlated with asparagine depletion in serum under both treatments. The non-inferiority of asparagine depletion under the recombinant asparaginase treatment compared to Asparaginase medac treatment was shown with the upper 95% confidence limit in % of LS means of reference exceeding Δ = -20.

**STUDY MC-ASP.2/RHN**

Objective

The purpose of this single-centre, uncontrolled, phase I/II study was to evaluate the pharmacokinetics, pharmacodynamics, efficacy and safety of recombinant L asparaginase in adult patients with relapsed/refractory haematological neoplasias.

Primary endpoint

Area under the plasma concentration versus time curve (AUC) from end of first infusion (time point: 0 h) up to 72 hours ($AUC_{0-72}$).

Secondary endpoints

Elimination half-life ($t_{1/2}$), trough levels before subsequent infusions, plasma concentrations of the amino acids asparagine (ASN), aspartic acid (ASP), glutamine (GLN), and glutamic acid (GLU), correlation of

ASN concentration to L-asparaginase activity over time, efficacy by reduction of peripheral blasts and/or decrease of LDH and/or decrease of paraproteinaemia, adverse events

Overall Study Design

The study was open labelled, non-randomised, non-controlled.

A sample size of 14 adult patients with relapsed/therapy-refractory or indolent haematological neoplasias (AML, MDS, NHL) was planned for assignment to treatment with the investigational medicinal product (IMP). The total duration of treatment was planned to be 36 days at the most for an individual patient.

All patients were to receive up to a maximum of 8 recombinant L-asparaginase infusions. The patients received study medication every third day, ending with the eighth infusion on day 22 at the latest (i.e. treatment days 1, 4, 7, 10, 13, 16, 19, and 22). Recombinant L-asparaginase was administered as an intravenous (IV) infusion over 30 minutes at a dose of 5,000 U/m² (calculated by the treating physician). During and up to 7 days after treatment with recombinant asparaginase, the administration of other antineoplastic agents was not permitted to avoid possible drug interactions; although there were no restrictions concerning prior therapy, diet or other supportive medication.

The administration interval and dosing of recombinant L-asparaginase was based on that of the older medac product. The dose selected was that used in the induction phase of current paediatric ALL treatment where native L-asparaginase preparations are scheduled. In contrast to the current ALL treatment protocols, L-asparaginase was administered as monotherapy in this pilot study. Dosage modifications were not necessary and not allowed. The principal investigator decided case by case about the number of L-asparaginase doses each patient received.

Study Population

*Main inclusion criteria*

- AML ≥ 2nd relapse or therapy-refractory (except acute promyelocytic leukaemia [FAB M3])
- MDS ≥ 1st relapse or therapy-refractory
- NHL ≥ 3rd relapse or therapy-refractory or indolent
- Smouldering disease, defined as no urgent need for aggressive multi-agent chemotherapy

*Main exclusion criteria*

- Pre-treatment with any L-asparaginase preparation
- General health status according to WHO score (ECOG) < 3
- Pre-existing coagulopathy (e.g. haemophilia; Quick test < 70%)
- Pre-existing pancreatitis
- Kidney, Liver, Haematological insufficiency *(leukocytes< 2/nl; thrombocytes< 50/nl)*

Pharmacokinetic measurements

Blood samples for PK measurements were collected at specific pre-defined time points before start (-½ h) and after end of the first infusion (0, ¼, ½, 1, 2, 4, 6, 24, 30, 48, 54, and 72 h), as well as always immediately before subsequent recombinant L-asparaginase doses, and 14 days after the last infusion had been administered.

From the L-asparaginase activities measured in plasma after the first infusion, at least the following pharmacokinetic parameters were determined using the actual blood sampling times for calculation within a non-compartmental model:

- $AUC_{0-72}$: Area under the plasma concentration versus time curve (AUC) from end of infusion (time point: 0 h) up to 72 h thereafter (time point: 72 h) Unit: [amount (U) · time (h)/volume(L)] determined by the log-linear trapezoidal formula
- $t_{1/2\lambda z}$: Apparent terminal elimination half-life: t1/2λz = ln(2) / λz Unit: [time (h)] where λz denotes the terminal rate constant estimated by linear regression analysis from a range of concentrations, which by inspection appear to be on a straight line in the logarithmic-linear plot

***Results***

Patient disposition

The trial was prematurely discontinued in November 2005 due to non-compliance with the time schedule as only 8 of the 14 planned patients were enrolled in 23 months instead of the projected 6 months. The first patient never received therapy with the study drug because of thrombocytopenia after registration. None of the other seven patients was treated with the full course of maximum 8 doses (varied between 1 and 5 infusions); 4 had treatment discontinued due to low anti-thrombin III levels because of risk of development of thromboembolic complications.  Due to the palliative character of the study, there was reluctance to accept decreased AT III levels to the magnitude of what is generally tolerated when treating patients with curative intent.

Analysis of pharmacokinetics

The individual courses of the plasma recombinant plasma L-asparaginase activities after the first infusion are presented as unfitted graphs in the following spaghetti plot.

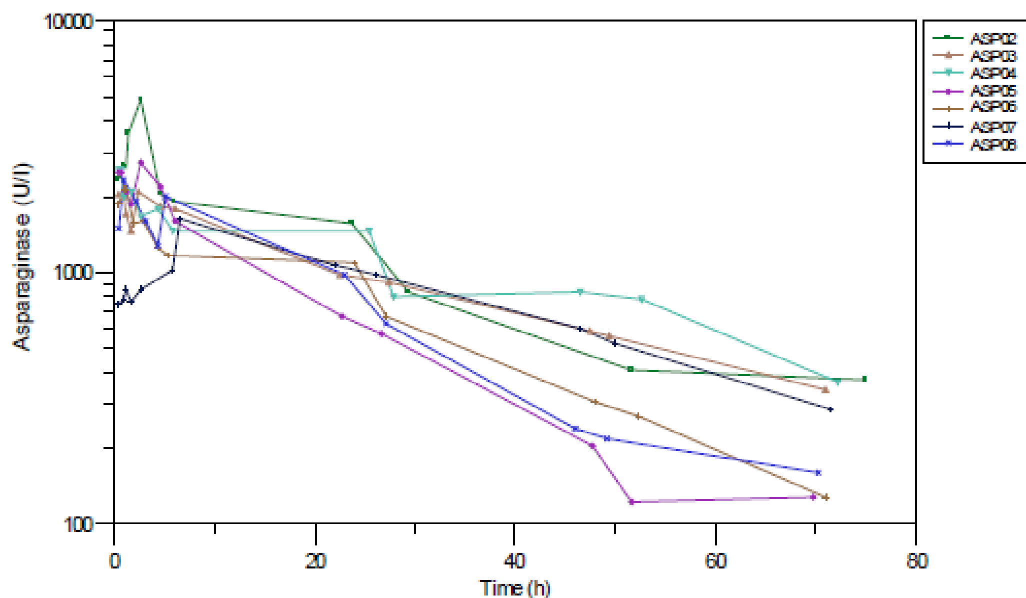



**Figure 22: Individual L-asparaginase AS activities versus time course after first administration (Study MC-ASP.2/RHN)**

From the L-asparaginase activities measured in plasma after the first infusion, the following individual pharmacokinetic parameters were computed with a non-compartmental model.

**Table 39: Individual PK parameters (Study MC-ASP.2/RHN)**

| Patient number | $c_{max}$ (U/L) | $t_{max}$ (h) | $AUC_{tot}$ (h · U/L) | $AUC_{0-72h}$ (h · U/L) | $t_{1/2}$ (h) | MRT (h) | Clearance (L/h) | $V_{ss}$ | $c_{last}$ (U/L) |
|---|---|---|---|---|---|---|---|---|---|
| 02 | 4819 | 2.62 | 89,732 | 78,275 | 26.0 | 33.7 | 0.06 | 1.88 | 305 |
| 03 | 2080 | 2.33 | 79,303 | 63,934 | 31.1 | 42.2 | 0.06 | 2.66 | 343 |
| 04 | 2558 | 0.43 | 84,170 | 73,495 | 20.1 | 36.7 | 0.06 | 2.18 | 368 |
| 05 | 2728 | 2.78 | 47,114 | 45,512 | 14.2 | 18.8 | 0.11 | 2.00 | 78 |
| 06 | 2151 | 0.93 | 50,614 | 47,177 | 18.4 | 26.7 | 0.10 | 2.64 | 129 |
| 07 | 1625 | 6.75 | 68,391 | 57,306 | 26.2 | 40.0 | 0.07 | 2.92 | 293 |
| 08 | 2324 | 1.05 | 61,972 | 51,726 | 44.2 | 37.2 | 0.08 | 3.00 | 161 |
| Mean | 2612 | 2.41 | 68,756 | 59,632 | 25.8 | 33.6 | 0.08 | 2.47 | 240 |
| SD | 1036 | 2.12 | 16,495 | 12,784 | 9.9 | 8.2 | 0.02 | 0.45 | 115 |

[1] $c_{max}$ = maximum plasm ASNase activity; $t_{max}$ = time to reach $c_{max}$; AUC = area under the ASNase activity-time curve; $t_{1/2}$ = half life; $V_{ss}$ = volume of distribution in plasma at steady state; $c_{last}$ = computed plasma ASNase activity 72 hours after infusion

The mean ± SD area under the plasma concentration versus time curve from end of first infusion to 72 h (primary endpoint) was 59,632 ± 12,784 h·U/L and ranged from 45,512 to 78,275 h·U/L. This result served as a comparator for a subsequently conducted bioequivalence study of recombinant L-asparaginase versus a commercially available asparaginase preparation in children with de novo ALL. The AUC and the half-life were comparable to the data established for other E.coli L-asparaginase preparations.

The peak activity ($C_{max}$) of L-asparaginase was reached with a delay of about 2 hours after the end of the infusion. The reason for the delay is unknown. In only one patient, the $C_{max}$ was obtained at the end of infusion. The apparent volume of distribution in plasma at steady state was 2.5 L, which is equivalent to the plasma volume.

Under treatment with L-asparaginase, the trough asparaginase activity was above the desired threshold level of >100 U/L. Fourteen days after the last infusion, L-asparaginase activities were below the lower limit of quantification (as shown below).

**Table 40: Trough recombinant L-asparaginase activities (U/L) in plasma after repeated dosing (Study MC-ASP.2/RHN)**

| Patient number | number of infusions | before infusion 2 | before infusion 3 | before infusion 4 | before infusion 5 | 14 days after last infusion |
|---|---|---|---|---|---|---|
| 02 | 3 | 378 | 311 | --- | --- | nd[1] |
| 03 | 5 | 342 | 510 | 393 | 175 | BLLQ[2] |
| 04 | 2 | 362 | nd[1] | --- | --- | nd[1] |
| 05 | 2 | 127 | 108 | --- | --- | nd[1] |
| 06 | 1 | 127 | --- | --- | --- | BLLQ[2] |
| 07 | 2 | 283 | 289 | --- | --- | nd[1] |
| 08 | 1 | 161 | --- | --- | --- | nd[1] |

[1] not done
[2] BLLQ = below lower limit of quantification, LLQ = 50 U/L

The ASN concentrations rapidly dropped from the pre-treatment value of 47.8 ± 15.0 μM to below the limit of detection of the bioanalytical method at nearly all measured time points in all patients under treatment with L-asparaginase.

**Table 41: Individual concentrations of ASN in plasma (µM) (Study MC-ASP.2/RHN)**

| Patient number<br>Time point | 02 | 03 | 04 | 05 | 06 | 07 | 08 | Mean ; SD |
|---|---|---|---|---|---|---|---|---|
| before infusion 1 | 44.6 | 47.4 | 51.6 | 58.2 | 30.5 | 72.2 | 30.0 | 47.8 ; 15.0 |
| 0 h[2] | < 0.5 | < 0.5 | 1.7 | 1.5 | < 0.5 | < 0.5 | < 0.5 | 0.6 ; 0.7 |
| ¼ h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| ½ h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| 1 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 1.1 | < 0.5 | 0.4 ; 0.3 |
| 2 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 1.9 | 0.5 ; 0.6 |
| 4 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| 6 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 1.4 | < 0.5 | 0.4 ; 0.4 |
| 24 h | < 0.5 | < 0.5 | 0.7 | < 0.5 | 0.8 | < 0.5 | < 0.5 | 0.4 ; 0.2 |
| 30 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| 48 h | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| 54 h | nd[4] | nd | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.3 ; 0.0 |
| 72 h[3] | < 0.5 | < 0.5 | 1.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | 0.4 ; 0.5 |
| before infusion 3 | < 0.5 | < 0.5 | nd | < 0.5 | --- | < 0.5 | --- | 0.3 ; 0.0 |
| before infusion 4 | --- | < 0.5 | --- | --- | --- | --- | --- | nc[5] |
| before infusion 5 | --- | < 0.5 | --- | --- | --- | --- | --- | nc |
| 14 days after last infusion | nd | < 0.5 | nd | nd | 4.7 | nd | nd | nc |

[1] LLQ: 0.5 µM (see section 9.5.2). Values below LLQ were calculated as half of LLQ (see section 9.7.1)
[2] immediately after end of infusion 1
[3] before infusion 2
[4] not done
[5] not calculated because of limited measurements / missing values

The ASN depletion in plasma was 98.7% immediately after end of infusion number 1 (time point 0 h) and remained above 99% in all patients under treatment until the final examination.

A distinct long-lasting increase in ASP concentrations was observed from immediately after end of the first infusion (time point 0 h) until three days thereafter. In patients receiving more than one infusion, the ASP concentration remained elevated throughout the treatment and was still increased 14 days after the last infusion (data from only two patients available at that time point).

A slight decrease in GLN plasma concentrations within four hours after the first infusion was observed. Thereafter, GLN concentrations increased again returning to pre-treatment values at six hours after infusion and remaining comparable to those until 54 hours after the first infusion presumably due to increased endogenous production and/or exogenous supply of this amino acid. Before the second and the third infusion, GLN concentrations were elevated compared to baseline, but were slightly lower at the final examination.

The arithmetic mean GLU concentration in plasma markedly increased during treatment with recombinant L-asparaginase and returned to values comparable to those before treatment start 14 days after the last infusion.

Efficacy data

Efficacy of treatment was evaluated by measuring the % reduction of peripheral blasts, and/or the % decrease of LDH, and/or the % decrease of paraprotein (in patients with MM only) compared to the corresponding baseline values. In all but two patients, a decrease of LDH was observed ranging from 4.3% to 49.5%. The reduction of paraprotein was not determined because patients with MM were not included in the study. Apart from at baseline, peripheral blasts were not determined at any other time point during or after treatment in the AML patients. Therefore, the evaluation of efficacy with respect to reduction in peripheral blasts was not possible.

**Study MC-ASP.6/INF**

Study objectives

This non-controlled multicentre phase II study was designed to assess the safety and to describe (in relation to children of higher age) the pharmacodynamics of recombinant asparaginase for first-line treatment in infants (< 1 year of age at diagnosis) with de novo ALL.

Primary Endpoint

The number of patients with hypersensitivity reactions to recombinant asparaginase during induction treatment defined as:

- Any allergic reaction occurring during or up to 12 hours after recombinant asparaginase infusion (e.g. rash, urticaria, oedema/angioedema, symptomatic bronchospasm, anaphylaxis)
- Silent inactivation of asparaginase activity, defined as asparaginase trough serum activity < 20 U/L (directly before recombinant asparaginase administration numbers 2, 4, and 6).

Assessments of allergic reactions, silent inactivation and hypersensitivity reactions were made on Days 15, 18, 22, 25, 29 and 33.

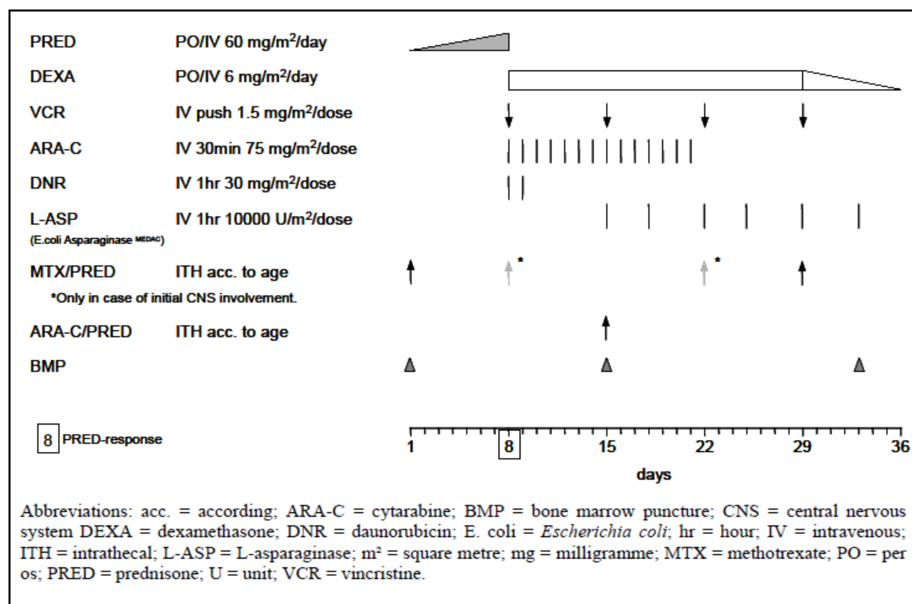
Secondary Endpoints

- trough level of asparaginase activity in serum just before the next recombinant asparaginase infusion
- concentrations of ASN, ASP, GLN, and GLU in serum directly before recombinant asparaginase infusions
- number of patients with complete ASN depletion in serum during induction treatment as defined by ASN level ≤ 0.5 µM (which is the LLOQ)
- number of patients able to complete their full course of asparaginase treatment during induction
- CR rate and MRD status after induction treatment phase according to the INTERFANT-06 protocol (Day 33 or thereafter)
- relapse rate, relapse-free survival and event free survival at the end of the follow-up (5 weeks after the last study patient received the last recombinant asparaginase infusion)
- anti-asparaginase antibodies (using the same assay as in the study MC-ASP.5/ALL)

Study Design

12 evaluable patients were included. Patients were treated according to the protocol INTERFANT-06 and received 6 doses of asparaginase during the induction treatment.

**Table 42 Induction Phase of the INTERFANT-06 Protocol**




Abbreviations: acc. = according; ARA-C = cytarabine; BMP = bone marrow puncture; CNS = central nervous system DEXA = dexamethasone; DNR = daunorubicin; E. coli = *Escherichia coli*; hr = hour; IV = intravenous; ITH = intrathecal; L-ASP = L-asparaginase; m² = square metre; mg = milligramme; MTX = methotrexate; PO = per os; PRED = prednisone; U = unit; VCR = vincristine.

Patients received recombinant asparaginase instead of asparaginase medac. The treatment dose was 10,000 U/m², adjusted to the current age of the patient at the time of administration (Children < 6 months: 2/3 of the calculated dose; Children 6 through 12 months: 3/4 of the calculated dose) administered on Days 15, 18, 22, 25, 29, 33 of the INTERFANT-06 induction phase. The recombinant asparaginase was given as IV infusions over 1 ± 0.5 hours.

Patients

*Inclusion criteria*

- Previously untreated T-lineage or precursor B-lineage ALL or biphenotypic leukaemia according to European Group for the Immunological Characterization of Leukaemias (EGIL) 14 criteria.
- Morphological verification of the diagnosis, confirmed by cytochemistry and immunophenotyping.
- Age < 1 year at diagnosis.

*Exclusion Criteria*

- Mature B-lineage ALL, defined by the immunophenotypical presence of surface immunoglobulins or t(8;14) and breakpoint as in B-ALL.
- The presence of the t(9;22)(q34;q11) or bcr-abl fusion in the leukaemic cells
- Systemic use of corticosteroids less than 4 weeks before diagnosis.
- Pre-existing known coagulopathy, pancreatitis, liver insufficiency

***Results***

**Recruitment**

The first patient was randomised for study participation on 12-Aug-2009. The last visit of the last patient was performed on 14-Sep-2010.

Disposition of patients

Out of 12 patients, 8 completed the trial alive without relapse and 4 terminated the trial due to relapse.

Demographic and other baseline characteristics

Diagnosis of ALL was confirmed in the peripheral blood in 7 infants, a BM aspirate was performed in 5 infants. At time of diagnosis a median of 87.5% of leukaemic blasts in the peripheral blood was determined in all 12 patients. Assessment of Bone Marrow showed a median of 92.0% leukaemic blasts.

**Table 43: Summary of demographic data and disease characteristics (Study MC-ASP.6/INF)**

| Number of patients | 12 (100%) |
|---|---|
| **Age at first infusion [months]** | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 5.6 (4.0) |
| Median (Q1, Q3) | 6.0 (1.5, 9.0) |
| Min, Max | 0.5, 12.2 |
| **Age at first infusion (categorised)** | |
| <6 months | 6 ( 50%) |
| 6- ≤12 months | 5 ( 42%) |
| >12 months | 1 ( 8%) |
| **Height [cm]** | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 64.5 (9.6) |
| Median (Q1, Q3) | 65.5 (58, 72) |
| Min, Max | 46, 79 |
| **Weight [g]** | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 6861.3 (2203.9) |
| Median (Q1, Q3) | 6982.5 (4910, 8650) |
| Min, Max | 3100, 9870 |
| **Body Surface Area [m²]** | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 0.35 (0.09) |
| Median (Q1, Q3) | 0.36 (0.27, 0.43) |
| Min, Max | 0.19, 0.45 |
| **Sex** | |
| Female | 5 ( 42%) |
| Male | 7 ( 58%) |
| **Ethnicity** | |
| Black | 1 ( 8%) |
| White | 11 ( 92%) |

Source: Listing 16.2.4D

| Number of patients | 12 (100%) |
|---|---|
| Time between first ALL-diagnosis and start of INTERFANT-06 [days] | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 0.6 (1.0) |
| Median (Q1, Q3) | 0.0 (0, 1) |
| Min, Max | 0, 3 |
| Immunophenotype | |
| Pro-B-ALL | 9 ( 75%) |
| Common ALL | 1 ( 8%) |
| Pre-B-ALL | 2 ( 17%) |
| Genetics | |
| *MLL*-AF4 | 5 ( 42%) |
| *MLL*-AF9 | 1 ( 8%) |
| *MLL-ENL* | 2 ( 17%) |
| Other: t(1,14) | 1 ( 8%) |
| No aberration | 3 ( 25%) |
| Peripheral blood-blasts [%] | |
| N | 12 |
| $N_{miss}$ | 0 |
| Mean (SD) | 74.0 (31.7) |
| Median (Q1, Q3) | 87.5 (68, 93) |
| Min, Max | 9, 98 |
| Bone marrow-blasts [%] | |
| N | 5 |
| $N_{miss}$ | 7 |
| Mean (SD) | 81.0 (16.6) |
| Median (Q1, Q3) | 92.0 (67, 93) |
| Min, Max | 60, 94 |
| Risk group stratification | |
| low risk | 4 ( 33%) |
| medium risk | 4 ( 33%) |
| high risk | 4 ( 33%) |
| Post induction treatment | |
| Experimental arm | 2 ( 17%) |
| Standard arm | 10 ( 83%) |

Source: Listings 16.2.4.A, 16.2.4.E and 16.2.6E

Efficacy evaluation

For results of the primary endpoint (number of patients with Hypersensitivity Reactions during Induction) please refer to the clinical safety section.

*Secondary endpoints:*

*Asparaginase Activity Levels in Blood Serum during Induction*

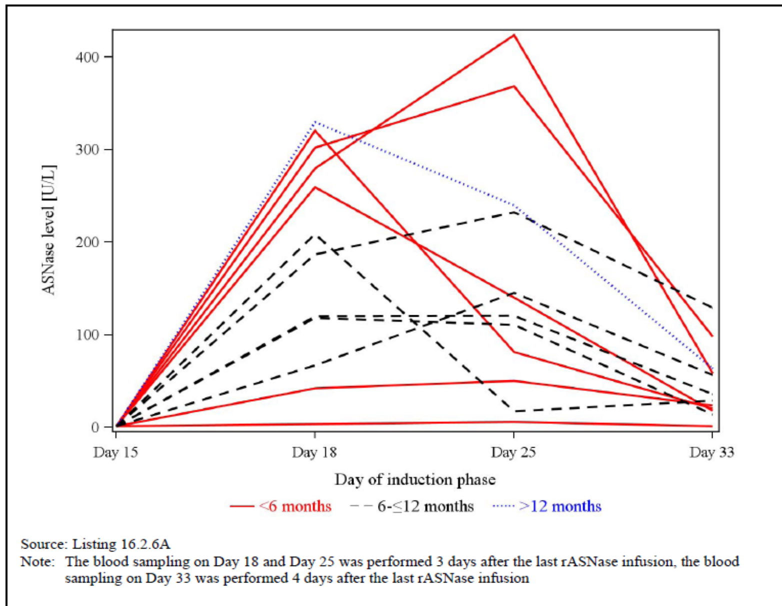



**Figure 23: Spaghetti Plot of Individual Serum Trough Asparaginase Levels versus Day of Induction (Study MC-ASP.6/INF)**

The variability of the serum trough asparaginase levels between patients was high. Median serum trough asparaginase levels were 208.810 U/L on Day 18, 130.160 U/L on Day 25 and 32.420 U/L on Day 33. The trough asparaginase levels on Day 33 were considerably lower as compared to Day 18 and Day 25 which was due to the fact that the latter levels were assessed 3 days after recombinant asparaginase infusion while assessment on Day 33 was performed 4 days after the last recombinant asparaginase infusion.

*Concentrations of Amino Acids in Serum during Induction*

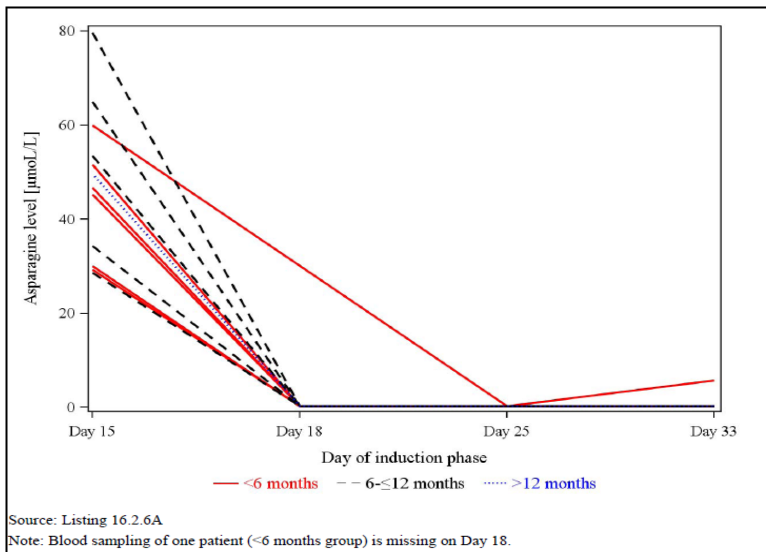



**Figure 24: Spaghetti Plot of Individual Asparagine Levels versus Day of Induction (Study MC-ASP.6/INF)**

**Table 44: Complete Asparagine Depletion during Induction (Study MC-ASP.6/INF)**

| | n (%) | Exact 90%-Confidence interval* |
|---|---|---|
| Number of patients | 12 (100%) | |
| Patients with complete ASN depletion | | |
| on Day 18 (before 2nd ASNase infusion)[a] | 11 (100%) | [ 76% ,100%] |
| on Day 25 (before 4th ASNase infusion) | 12 (100%) | [ 78% ,100%] |
| on Day 33 (before 6th ASNase infusion) | 11 ( 92%) | [ 66% ,100%] |
| on Day 18, Day 25 and Day 33 | 11 ( 92%) | [ 66% ,100%] |

Source: Listing 16.2.6A
* Pearson-Clopper Confidence interval
[a] Patient Number 06 excluded due to missing value

Only 1 patient had detectable levels of asparagine in serum at Day 33. The median baseline level of ASN was 48.155 µmoL/L (Min, Max 28.570, 79.710 µmoL/L).

The median aspartic acid level in serum was 3.18 µmoL/L at baseline. On Day 18 and Day 25 measurements before recombinant asparaginase infusion resulted in increased median ASP levels of 12.89 µmoL/L (Min, Max 5.10, 23.21 µmoL/L) and 14.90 µmoL/L (Min, Max 4.91, 40.11 µmoL/L), respectively. On Day 33 the median ASP level had decreased to 7.58 µmoL/L (Min, Max 3.74, 44.19 µmoL/L).

The median level of glutamine was 476.90 µmoL/L at baseline decreasing slightly from 397.78 µmoL/L on Day 18 to 379.40 µmoL/L on Day 25 and to 347.77 µmoL/L (Min, Max 217.29, 490.34 µmoL/L) on Day 33.

The median level of glutamic acid was 94.89 µmoL/L at baseline and stayed steady during induction, decreasing slightly to 74.17 µmoL/L on Day 33.

*Complete Remission Rate and Minimal Residual Disease Status after Induction*

**Table 45: Summary of CR and MRD Status after Induction (Study MC-ASP.6/INF)**

| | n (%) | Exact 90%-Confidence interval* |
|---|---|---|
| Number of patients | 12 (100%) | |
| CR | 12 (100%) | [ 78%, 100%] |
| MRD status | | |
| ND | 2 ( 17%) | |
| negative | 1 ( 8%) | |
| positive | 9 ( 75%) | |

Source: 16.2.6E
* Pearson-Clopper Confidence interval

*Leukaemic Blast*s

On Day 33 all patients showed a percentage of below 5% of leukaemic blasts in BM. The median percentage of leukaemic blasts in BM was 0.5% (Min, Max 0, 3.0%).

*Relapse Rate and Event-Free Survival*

The relapse rate and EFS were evaluated at the end of the follow-up which ended 5 weeks after the last patient received the last recombinant asparaginase infusion. Four patients experienced a relapse - cumulative incidence: 27% after 6 months (90% CI [6%, 49%]) and 42% after 12 months (90% CI [16%, 68%]). The Kaplan Meier estimates of EFS were 73% after 6 months (90% CI [51%, 94%]) and 58% after 12 months (90% CI [32%, 84%]).

**STUDY MC-ASP.1/ALL**

Study design: Open labelled, non-randomised, non-controlled, multicentre, phase II study.

Objectives:

To evaluate the pharmacokinetics, pharmacodynamics, efficacy, and safety of repeated administrations of recombinant asparaginase (recombinant asparaginase) in adult patients with relapsed acute lymphoblastic leukaemia (ALL)

- number of patients with complete asparagine depletion during induction treatment
- trough and peak levels of asparaginase
- serum concentrations of the amino acids asparagine, aspartic acid, glutamine, glutamic acid
- complete remission

Main Criteria for Inclusion

- adults with first relapse of acute lymphoblastic leukaemia
- age ≥ 18 to ≤ 60 years
- no pre-existing coagulopathy, pancreatitis, kidney and/or liver insufficiency

Patients

Number of patients planned: 15

Number of patients included: 2 (22 yr old female/ 59 yr old male)

Number of patients analysed: 2

Treatment

Patients enrolled in this study were to undergo treatment for ALL relapse according to the GMALL (German Multicenter ALL Study Group (for adults)) relapse protocol ALLREZ 02/92. In this protocol, the use of 10,000 U/m$^2$ per dose of a native asparaginase is scheduled. For this study, however, the native asparaginase is replaced with the new recombinant product at the same dose.

Patients received 4 infusions at study days 7, 8, 14, and 15 within a total study duration of 24 days.

***Results***

The study was terminated after enrolment of two patients due to very poor recruitment.

Pharmacokinetic results

The trough asparaginase activity in serum was mostly above the desired threshold level of 100 U/L.

Pharmacodynamic and efficacy results

The patients showed complete ASN depletion in serum. Minor short-time glutamine cleavage was shown. No systematic changes in aspartic acid and glutamic acid were observed.

In one patient, remission status after end of induction treatment could not be assessed due to numerous missing values for peripheral blood and bone marrow parameters. The second patient did not enter into a complete remission presenting with 10 % blasts in bone marrow after induction.

### 2.5.3. Discussion on clinical efficacy

**Design and conduct of clinical studies**

Recombinant asparaginase (Spectrila) has been studied in two main trials: one pivotal phase III (MC-ASP.5/ALL) and one phase II (MC-ASP.4/ALL). In both trials, recombinant asparaginase was compared to asparaginase medac. The MC-ASP.4/ALL and MC-ASP.5/ALL trials had a common study design (randomised, double-blind, asparaginase medac-controlled), with similar endpoints (pharmacodynamics and efficacy), and selected target populations (de novo ALL children $\geq$ 1 $\leq$ 18 years old).

Against what was recommended by the CHMP (EMEA/CHMP/SAWP/164920/2005), the Applicant did not perform a dose-finding study. A preliminary dose selection would have been preferable, based on the high species-specific variability and the PK/PD differences observed between asparaginase medac and recombinant asparaginase in non-clinical studies (see non-clinical section).

The CHMP recommended that the applicant demonstrate the pharmacodynamic equivalence of Asparaginase medac and recombinant L-asparaginase, using suppression of asparagine concentrations as a measure (EMEA/CHMP/SAWP/164920/2005). The Applicant was also advised to provide adequate reassurance for the validity of the asparagine assay, in particular, the potential for asparaginase to interfere with the sensitivity of the assay. Supportive evidence of pharmacological equivalence was expected to be provided, specifically for the measures: intracellular concentration of asparagine, plasma asparaginase activity and suppression of asparagine concentration in CSF. Data showing the adequate suppression of serum asparagine concentrations after repeat dosing was requested in order to confirm that efficacy is maintained with repeat dosing. The protocol assistance was in general followed except for the evaluation of intracellular concentration of asparagine which was not conducted based on literature data showing that no changes in intracellular levels of twenty amino acids including ASN could be measured in leukaemic cells *in vivo* after treatment with a single dose of pegylated asparaginase (*Appel et al.)*. The lack of data on intracellular asparaginase is not expected to significantly impact the benefit-risk of the product.

With regards to PK evaluation, the Applicant used the microplate reader-based method (AHA-assay) for measuring asparaginase activity in serum, plasma and CSF. Asparaginase activity rather than amount of protein was measured which is acceptable. The validation of the assay is also acceptable. However, during the evaluation of the dossier, the EMA was alerted of previous GCP non-compliance detected at the laboratory site which was involved in the conduct of the two mains studies MC-ASP.4/ALL and MC-ASP.5/ALL and therefore a GCP triggered inspection was requested. Several findings were identified during the inspection, including one critical finding within the laboratory site concerning the asparaginase assay in study MC-ASP.5/ALL. Specifically, the finding was that of non-adherence to the study plan criteria for accepting or rejecting calibration standards for the asparaginase assay. Therefore, some batches which should have failed were passed, whilst one which should have passed was failed. Recalculations of the asparaginase data were provided, using the methods outlined in the study plan. Applying the more stringent analysis method (method I) the number of non-valid samples was considerably increased compared to the original method; although a fair proportion occurred on Day 12, i.e. baseline. Data from this re-analysis showed that the mean asparaginase activity differences between the study arms at the later study time points were slightly larger than initially shown (Asparaginase medac > recombinant asparaginase) with data based on the original analysis method (see efficacy analysis). The trend towards increasing size of the difference between the proportion of subjects in the test and reference arms with samples registering <100U/L of asparaginase activity as the study progressed appeared to be maintained within the analysis of method I samples. These differences were seen with the originally presented data and were of similar magnitude; with no suggestion that the method I analyses (unlike the other PK analyses) result in larger differences between test and reference

product. Likewise, the results of the method I analyses of samples with asparaginase activity < and ≥ 100 U/L did not differ significantly from the originally presented analyses.

Regarding pharmacodynamic parameters, the Applicant described in detail the methods for the detection of asparagine in human serum and CSF. The Applicant also discussed data from a number of ex-vivo experiments and publications which rather suggested that even at low temperatures (2-8◦C) and with a range of activity levels, asparaginase hydrolysis of asparaginase still occurred with very high velocity. Overall, whilst acknowledging that there appears not to be an optimal method for inhibiting asparaginase activity immediately after venepuncture, consequently halting ex-vivo hydrolysis of asparagine, it is considered that appropriate steps were taken to halt asparaginase activity as soon as practicably possible after venepuncture. The assay was also appropriately validated for its intended purpose.

**Efficacy data and additional analyses**

The pharmacodynamic and pharmacokinetic parameters of complete asparagine depletion and serum asparaginase activity are considered the main outcome measures provided in support of comparable efficacy between the test and the comparator. Efficacy outcomes are only considered supportive in this development programme.

The pharmacokinetics of recombinant asparaginase versus native asparaginase was evaluated after intravenous infusion of a first dose in 30 children with acute lymphoblastic leukaemia in study MC-ASP.4/ALL. Additionally, trough levels were evaluated in serum during induction treatment in studies MC-ASP.1/ALL, MC-ASP.5/ALL, MC-ASP.6/INF and MC-ASP.4/ALL. A limited number of adult patients were evaluated. Unfortunately, the adult studies MC-ASP.1/ALL and MC-ASP.2/RHN were terminated early due to poor recruitment with only 10 adult patients enrolled. The applicant will conduct a post-approval study evaluating PK, PD and safety (incl. immunogenicity) in adult ALL patients as reflected in the RMP (additional pharmacovigilance activity). This study will enable to generate further efficacy data in adult patients.

In study MC-ASP.4/ALL where the equivalence of asparaginase medac and recombinant asparaginase was evaluated using $AUC_{0-72h}$, a statistically significant difference was seen between the two study arms (p=0.020). The point estimate of the $AUC_{0-72h}$ for the test treatment was lower than that of the reference product, with a point estimate of the treatment ratio of 86.01. The confidence intervals of the geometric mean ratio between the groups fell within the acceptance limits set. However, these limits had been widened from the usual 80-125 % bracket to 75-133% leading to uncertainties regarding the bioequivalence between recombinant asparaginase and asparaginase medac.

From the trough measurements provided, it appears that activity for recombinant asparaginase may be up to 20% lower than that of asparaginase medac. The higher serum activities could possibly be due to the higher levels of aggregates seen in the asparaginase medac product (20.5%) compared to recombinant asparaginase (approx. 0.3%). In the larger study MC-ASP.5/ALL, a trend to greater asparaginase activity, which increased towards the end of the induction treatment period was seen. This effect possibly results from the longer half-life of aggregates, which progressively increases activity of asparaginase medac in comparison to the relatively constant level of serum activities in the recombinant asparaginase group. The size of the differences in trough activities in the latter half of the dosing period were further increased by the method I analysis.

With regard to activity measurements in the subgroups of infants and adults, a proportion of patients experienced decreases in asparaginase activity with repeated administration. There is no comparison to asparaginase medac in these subgroups and in general data collection was patchy as a significant number of patients withdrew before study completion for a number of adverse events and experienced silent inactivation of asparaginase. However, considering the experience gained with the widespread use of asparaginases in treatment protocols for these groups it is considered that efficacy and safety of Spectrila

in toddlers, children and adolescents, can be extrapolated to these subgroups. Additional data will also be available from the planned post-approval study evaluating PK, PD and safety (incl. immunogenicity) in adult ALL patients (see RMP).

Some literature data (primarily non-clinical) suggest that a trough asparaginase activity of >100U/L is required for effective clearance of asparagine from plasma and CSF. However, data presented by the applicant for both studies MC-ASP.4-ALL and MC-ASP.5-ALL (PK/PD graphs not shown) showed that even asparaginase activities as low as 10U/L were associated with complete asparagine depletion in serum. This was also observed in the infant study. Data on paired serum asparaginase and CSF asparagine samples were provided, showing that even where patients failed to maintain serum asparaginase activities above 100U/L, asparagine depletion in CSF was complete.

Data from all clinical studies with recombinant asparaginase showed swift, sustained and complete reductions in serum and CSF asparagine. The primary endpoint in the pivotal study, MC-ASP.5 /ALL, was the pharmacodynamic endpoint of complete asparagine depletion. This was accepted as a valid surrogate for clinical efficacy of asparaginases. The non-inferiority/equivalence margin for the comparison of recombinant asparaginase to asparaginase medac was set at 10%. As the confidence intervals for the risk difference in both the per protocol set and full analysis set fall entirely within [-10%, 10%], the study was considered to have met its primary endpoint. Statistically the non-inferiority margin was adequately justified in line with EMA guidance on selecting a non-inferiority margin, with a substantial fraction of the asparaginase medac effect over placebo (at least 89.5%) preserved with recombinant asparaginase treatment. However, the margin was less well justified clinically and it was unclear that a 10% difference in the proportion of patients with complete asparagine depletion would not lead to a clinically relevant difference in efficacy. Nevertheless, it was noted that the point estimates showed the proportions to be nearly equal.

Further supportive evidence of efficacy was sought through comparison of data for other PD and PK endpoints which would reflect depth of asparagine depletion and cumulative exposure to enzyme activities known to be associated with pronounced anti-leukemic effects. These endpoints included duration of asparagine depletion, time to asparagine recovery (to pre-study levels), proportion of patients (and samples) with asparaginase activity below 100U/L and duration of asparaginase activity above 100 U/L. Small and non-statistically significant differences favouring asparaginase medac in duration of ASN depletion in serum, proportion of patients with ASNase activity >100 U/L, and proportion of samples with ASNase activity in serum <100 U/L during indication phase from day 15 to day 33 of protocol were reported. Minor differences between rASNase and ASNase Medac in Study MC-ASP.4/ALL were also observed with regard to asparagine recovery results. However, there were significant limitations associated with the submitted post-hoc analyses whcih undermine the robustness of the data; including sparse PK and PD sampling after the final adminsitation of asparaginase, a number of missing estimates and the small sample size in in Study MC-ASP.4/ALL. Given the limitations these data cannot be considered conclusive and should be interpreted with caution.

Increases in aspartic acid, a degradation product of asparagine were seen as expected in both study arms, although greater increases were seen in the asparaginase medac study arms. Changes in the levels of the other amino acids monitored did not reveal any notable differences between the two study arms.

The clinical relevance of the observed differences in PK and PD endpoints was discussed. The Applicant noted that duration of ASN depletion after last ASNase infusion and recovery of ASN to baseline value has been described as relevant endpoints in previous clinical trials with native ASNase preparations. In addition, ASN depletion in serum and CSF being the main surrogate for efficacy were considered more important than ASNase activity levels. Asparagine levels decreased after the first dose of either ASNase preparation and remained depleted until end of ASNase treatment. Although lower ASNase activity was observed for Spectrila versus ASNase medac the company argued that ASNase trough serum activity

levels as low as 10-20 U/L led to complete ASN depletion in serum and CSF based on the provided data and that supporting literature data show that lower levels than 100 IU/L are sufficient.

Some clinical efficacy measures were evaluated in the comparative clinical studies e.g. time to event measures, response rate and overall survival. For these outcomes there was very little difference between the study arms in the larger comparative trials. This was expected as the response rates in children with ALL enrolled on current induction treatment protocols normally show very good improvement and often remission. The assay sensitivity of these endpoints is considered low, as the response rates are usually exceptional and as a small number of events occur within clinical trials of reasonable length.

Complete remission rates are in line with what would be expected with this type of multi-agent induction treatment regimen. Minor differences are reported between the two arms in both studies. However, the number of patients included in the MC-ASP.4/ALL study is too small for interpretation of the data, and the differences between arms in the MC-ASP.5/ALL study could be partially influenced by an imbalance in NE and NK cases between the treatment arms.

Similar efficacy in terms of relapse- and event-free survival (RFS and EFS) was observed between recombinant asparaginase and asparaginase medac. Analysis of clinical efficacy parameters by genetic subtype (no aberration, Hyperdiploid, BCR/ABL, TEL/AML1, MLL-AF4), immunophenotype (common ALL, pre-B ALL, pre-pre B ALL, T-ALL) and disease characteristics was undertaken and showed no major differences between treatment arms or subgroups; although a number of these groups contained limited numbers of patients.

The efficacy of recombinant asparaginase and incidence of hypersensitivity reactions (see clinical safety) were also assessed in 12 infants patients (≤ 1 year) in the uncontrolled MC-ASP.6/INF study. Silent inactivation was reported in 4 out of the 12 enrolled patients (33%), even though no ADAs were detected in any patients. Nonetheless, complete ASN depletion was obtained in all but one subject. After induction treatment all patients were in CR and all but one achieved positive MRD status.

Data from the intensification stages of treatment are limited as only a small number of patients continuing asparaginase medac or recombinant asparagine treatment were enrolled into the high-risk (HR) group. The size of these subgroups makes it difficult to interpret the data submitted for that period. However the experience with the use of asparaginases in the post-induction phases in ALL treatment protocols is large. Therefore, if the efficacy and safety of Spectrila in induction treatment is justified and its use in post-induction treatment phases does not need to be restricted.

Efficacy of Spectrila has not been established in Philadelphia chromosome-positive patients (see SmPC section 4.4).

The sought indication for Spectrila included both acute B/T cell lymphoblastic leukaemia (ALL) and B/T cell lymphoblastic lymphoma (LBL). However, no B/T cell LBL patients have been treated with recombinant asparaginase in the submitted clinical trials. LBL is rare and has a very similar treatment strategy (i.e., chemotherapy regimens) to ALL. Therefore, a more concise indication referring to the treatment of acute lymphoblastic leukaemia in paediatric patients from birth to 18 years and adults is recommended. The LBL patient group is considered covered by this indication.

## 2.5.4. Conclusions on the clinical efficacy

Non-inferiority of Spectrila to asparaginase medac is considered shown on the basis of asparaginase depletion. Although PK differences between Spectrila and asparaginase medac were observed, these were not considered clinically significant. Further, the data showed that much lower serum asparaginase activities still resulted in complete asparagine depletion. Efficacy data will be available in adults as PK, PD,

safety and immunogenicity data will be collected post-approval in a single-arm adult de novo ALL patients study (see RMP).

## 2.6. Clinical safety

### Patient exposure

In total, 135 patients have been treated with recombinant asparaginase within five clinical studies sponsored by medac. The proposed standard administration schedule of recombinant asparaginase is 5,000 – 10,000 U/m$^2$ b.w. given intravenously every three days. The following tables list the dose regimen, duration of treatment, and maximum total dose of recombinant asparaginase within 5 clinical trials.

**Table 46: Dose regimen and duration of treatment with rASNase**

| Study | Dose | Induction treatment regimen | Post-induction treatment regimen |
|---|---|---|---|
| MC-ASP.2/RHN | 5,000 U/m$^2$ | q3d x maximal 8 | - |
| MC-ASP.1/ALL | 10,000 U/m$^2$ | Days 7, 8, 11, and 15 | - |
| MC-ASP.4/ALL | 5,000 U/m$^2$ | q3d x 8 | - |
| MC-ASP.5/ALL | 5,000 U/m$^2$ | q3d x 8 | - |
| | 10,000 U/m$^2$ | - | Days 22, 25, 29, and 32 of HR blocks 1, 2, 4, and 5;<br>Days 8, 11, 15, and 18 of protocol II |
| MC-ASP.6/INF* | 10,000 U/m$^2$ | Days 15, 18, 22, 25, 29 and 33 | - |

* The rASNase dose was individually adjusted by protocol to 67% of the calculated dose for infants < 6 months and to 75% of the calculated dose for infants aged 6-12 months.

**Table 47: Number of patients treated with rASNase per dose group**

| Dose | Route | Number of patients treated | | |
|---|---|---|---|---|
| | | Adults | Children (1 – 18 years | Infants (< 1 year) |
| 5,000 U/m$^2$ | IV | 7 | 114 | - |
| 6,670 U/m$^2$ | IV | - | - | 6 |
| 7,500 U/m$^2$ | IV | - | - | 5 |
| 10,000 U/m$^2$ | IV | 2 | 8* | 1 |

*Re-induction treatment. All 8 patients had also received a dose of 5,000 U/m$^2$ during induction treatment*

*Study MC-ASP.5/ALL: Extent of Exposure*

**Table 48: Summary of Study Drug Exposure during Induction Phase - Safety Analysis Set (Study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| Number of patients | 101 (100.0%) | 97 (100.0%) | 198 (100.0%) |
| Number of study drug infusions | | | |
| 1 | 2 ( 2.0%) | 0 (0%) | 2 ( 1.0%) |
| 2 | 1 ( 1.0%) | 1 ( 1.0%) | 2 ( 1.0%) |
| 3 | 1 ( 1.0%) | 0 (0%) | 1 ( 0.5%) |
| 5 | 2 ( 2.0%) | 1 ( 1.0%) | 3 ( 1.5%) |
| 6 | 1 ( 1.0%) | 3 ( 3.1%) | 4 ( 2.0%) |
| 7 | 0 (0%) | 3 ( 3.1%) | 3 ( 1.5%) |
| 8 | 94 ( 93.1%) | 89 ( 91.8%) | 183 ( 92.4%) |
| Duration of study drug treatment [days] | | | |
| Mean (SD) | 21.1 (3.9) | 21.6 (2.1) | 21.3 (3.2) |
| Median (Q1, Q3) | 22.0 (22.0, 22.0) | 22.0 (22.0, 22.0) | 22.0 (22.0, 22.0) |
| Min, Max | 1, 25 | 4, 23 | 1, 25 |
| Mean absolute dose of study drug administered per infusion during induction phase [U] | | | |
| Mean (SD) | 4534.8 (1930.8) | 4771.6 (1901.7) | 4650.8 (1915.4) |
| Median (Q1, Q3) | 3700.0 (3100.0, 5500.0) | 4200.0 (3250.0, 5750.0) | 4000.0 (3100.0, 5700.0) |
| Min, Max | 1900, 9500 | 2200, 10600 | 1900, 10600 |
| Switch of study drug | | | |
| No | 99 ( 98.0%) | 97 (100.0%) | 196 ( 99.0%) |
| Switch to Oncaspar | 2 ( 2.0%) | 0 (0%) | 2 ( 1.0%) |

Source: Listing 16.2.5A

During post-induction phase, the median number of study drug infusions was 3.5 (1 – 8) in the asparaginase medac treatment group and 8.0 (1 - 20) in the recombinant asparaginase treatment group. The median duration of IP treatment was 3.57 weeks (0.1 – 8.6 weeks) in the asparaginase medac group and 8.00 weeks (0.1 – 39.3 weeks) in the recombinant asparaginase group. Only 1 patient (administered recombinant asparaginase) proceeded to the High Risk 4 block and beyond.

*Study - MC-ASP.4/ALL: Extent of exposure*

Doses ranged from 2,400 U per dose to 9,400 U per dose, corresponding to a total of 19,200 U and 75,200 U, respectively, for eight infusions. One patient discontinued prematurely and only received the first seven infusions; all other patients received all eight scheduled asparaginase infusions.

*Study MC-ASP.2/RHN: Extent of exposure*

Seven patients received at least one dose of recombinant L-asparaginase (5,000 U/m²) and were evaluated for safety.

*Study MC-ASP.1/ALL: Extent of exposure*

Two patients were included in the safety analysis.

*Study MC-ASP.6/INF: Extent of exposure*

*Twelve patients* were included in the safety analysis.

**Adverse events**

- Study MC-ASP.5/ALL

Of the 101 patients receiving asparaginase medac, 96.0% experienced an AE and 33.7% experienced a drug-related AE during the course of the study. A total of 65 of these patients (64.4%) experienced an AE of at least CTCAE Grade III and 13.9% experienced a drug-related AE of at least CTCAE Grade III.

Of the 97 patients receiving recombinant asparaginase, 94.8% experienced an AE and 38.1% experienced a drug-related AE during the course of the study. A total of 58 of these patients (59.8%) experienced an AE of at least CTCAE Grade III and 19.6% experienced a drug-related AE of at least CTCAE Grade III. Serious AEs were experienced by 36.6% during the course of the study in the asparaginase medac group and 34.0% in the recombinant asparaginase treatment group.

**Table 49: Overall Summary of Adverse Events during Study - Safety Analysis Set (Study MC-ASP.5/ALL)**

| | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) |
| Number of patients | 101 | (100.0%) | 97 | (100.0%) | 198 | (100.0%) |
| Patients with AEs of any CTCAE Grade | 97 | ( 96.0%) | 92 | ( 94.8%) | 189 | ( 95.5%) |
| Patients with AEs of at least CTCAE Grade III | 65 | ( 64.4%) | 58 | ( 59.8%) | 123 | ( 62.1%) |
| Patients with drug related AEs of any CTCAE Grade | 34 | ( 33.7%) | 37 | ( 38.1%) | 71 | ( 35.9%) |
| Patients with drug related AEs of at least CTCAE Grade III | 14 | ( 13.9%) | 19 | ( 19.6%) | 33 | ( 16.7%) |
| Patients with at least one serious AE | 37 | ( 36.6%) | 33 | ( 34.0%) | 70 | ( 35.4%) |
| Results in death | 0 | (0%) | 3 | ( 3.1%) | 3 | ( 1.5%) |
| Life-threatening | 6 | ( 5.9%) | 5 | ( 5.2%) | 11 | ( 5.6%) |
| Hospitalisation or prolongation of hospitalisation | 33 | ( 32.7%) | 27 | ( 27.8%) | 60 | ( 30.3%) |
| Disability/Incapacity | 0 | (0%) | 0 | (0%) | 0 | (0%) |
| Congenital anomaly or birth defect | 0 | (0%) | 0 | (0%) | 0 | (0%) |
| Patients with drug related serious AEs | 16 | ( 15.8%) | 18 | ( 18.6%) | 34 | ( 17.2%) |
| Patients with maximum CTCAE Grade | | | | | | |
| CTCAE Grade I | 6 | ( 5.9%) | 8 | ( 8.2%) | 14 | ( 7.1%) |
| CTCAE Grade II | 27 | ( 26.7%) | 29 | ( 29.9%) | 56 | ( 28.3%) |
| CTCAE Grade III | 15 | ( 14.9%) | 10 | ( 10.3%) | 25 | ( 12.6%) |
| CTCAE Grade IV | 6 | ( 5.9%) | 5 | ( 5.2%) | 11 | ( 5.6%) |
| CTCAE Grade V | 0 | (0%) | 3 | ( 3.1%) | 3 | ( 1.5%) |

Source: Listing 16.2.7H, Listing 16.2.7I, Listing 16.2.7J, Listing 16.2.7K

**Table 50: Frequency of Patients with Asparaginase-Typical Adverse Events during all Periods of Treatment - Safety Analysis Set (Study MC-ASP.5/ALL)**

| ASNase-typical adverse events | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Number of patients | 101 | (100.0%) | 97 | (100.0%) | 198 | (100.0%) |
| Patients with any ASNase-typical adverse events | 57 | ( 56.4%) | 46 | ( 47.4%) | 103 | ( 52.0%) |
| Difference in Rates [%] (rASNase-ASNase medac) | | | -9.0% | | | |
| 95% CI[a] | | | [-22.85%; 5.15%] | | | |
| Allergic reaction within 12 hours after ASNase infusion | 11 | ( 10.9%) | 4 | ( 4.1%) | 15 | ( 7.6%) |
| Liver enzymes ( ≥CTCAE Grade III) | | | | | | |
| Any event | 41 | ( 40.6%) | 43 | ( 44.3%) | 84 | ( 42.4%) |
| ALT/GPT | 37 | ( 36.6%) | 42 | ( 43.3%) | 79 | ( 39.9%) |
| Hyperbilirubinemia | 11 | ( 10.9%) | 11 | ( 11.3%) | 22 | ( 11.1%) |
| AST/GOT | 7 | ( 6.9%) | 7 | ( 7.2%) | 14 | ( 7.1%) |
| Pancreatitis ( ≥CTCAE Grade II) | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Haemorrhage or thromboembolism ( ≥CTCAE Grade II) | | | | | | |
| Any event | 6 | ( 5.9%) | 2 | ( 2.1%) | 8 | ( 4.0%) |
| Thrombosis/thrombus/embolism | 2 | ( 2.0%) | 2 | ( 2.1%) | 4 | ( 2.0%) |
| CNS hemorrhage | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Hematoma | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Hemorrhage, GI [Oral cavity] | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Petechiae | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Neurotoxicity ( ≥CTCAE Grade III) | | | | | | |
| Any event | 6 | ( 5.9%) | 4 | ( 4.1%) | 10 | ( 5.1%) |
| Syncope (fainting) | 1 | ( 1.0%) | 3 | ( 3.1%) | 4 | ( 2.0%) |
| Seizure | 2 | ( 2.0%) | 0 | (0%) | 2 | ( 1.0%) |
| Dizziness | 0 | (0%) | 1 | ( 1.0%) | 1 | ( 0.5%) |
| Involuntary movement | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Mood alteration [Agitation] | 0 | (0%) | 1 | ( 1.0%) | 1 | ( 0.5%) |
| Neuropathy-motor | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |
| Somnolence | 1 | ( 1.0%) | 0 | (0%) | 1 | ( 0.5%) |

Source: Listing 16.2.7D, Listing 16.2.7E, Listing 16.2.7F, Listing 16.2.7G, Listing 16.2.7H, Listing 16.2.7I, Listing 16.2.7J, Listing 16.2.7K, Listing 16.2.8A
[a]unconditional exact confidence interval based on Chan and Zhang

The frequency of patients experiencing allergic reactions within 24 hours after ASNase infusion included all allergic reactions within 12 hours after ASNase infusion as defined in the study protocol plus all those adverse events with CTCAE terms syncope (fainting), hypotension, rash, flushing, pruritus, dyspnoea, injection site reaction or airway obstruction occurring within 24 hours after ASNase infusion. With this definition, allergic reactions during all periods of treatment with study drug occurred in 25 patients in the ASNase medac group (25%) and 17 patients in the rASNase group (18%).

*Hypersensitivity reactions and Anti-Asparaginase-Antibodies*

Comparative data on hypersensitivity reactions and silent inactivation are presented below.

**Table 51: Frequency of patients with modified hypersensitivity reactions, allergic reactions during or up to 12 hours after ASNase infusion and silent inactivations to the first dose of any asparaginase preparation in post induction phase – overall and stratified by risk group (SAS) (Study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Overall** | | | |
| Number of patients | 101 (100.0%) | 97 (100.0%) | 198 (100.0%) |
| Hypersensitivity reactions | | | |
| Yes | 39 ( 38.6%) | 37 ( 38.1%) | 76 ( 38.4%) |
| No | 51 ( 50.5%) | 49 ( 50.5%) | 100 ( 50.5%) |
| n.e. | 5 ( 5.0%) | 6 ( 6.2%) | 11 ( 5.6%) |
| NA | 6 ( 5.9%) | 5 ( 5.2%) | 11 ( 5.6%) |
| Difference[a] (rASNase - ASNase medac) | | -0.0% | |
| 95% CI[b] | | [-14.09%;14.03%] | |
| Allergic reaction within 12 hours after ASNase infusion | | | |
| Yes | 7 ( 6.9%) | 6 ( 6.2%) | 13 ( 6.6%) |
| No | 88 ( 87.1%) | 86 ( 88.7%) | 174 ( 87.9%) |
| NA | 6 ( 5.9%) | 5 ( 5.2%) | 11 ( 5.6%) |
| Silent inactivation | | | |
| Yes | 36 ( 35.6%) | 32 ( 33.0%) | 68 ( 34.3%) |
| No | 51 ( 50.5%) | 49 ( 50.5%) | 100 ( 50.5%) |
| n.e. | 8 ( 7.9%) | 11 ( 11.3%) | 19 ( 9.6%) |
| NA | 6 ( 5.9%) | 5 ( 5.2%) | 11 ( 5.6%) |

Source: Listing 16.2.7A, Listing 16.2.7B, Listing 16.2.7C, Listing 16.2.7D, Listing 16.2.7E, Listing 16.2.7F
[a] If hypersensitivity reaction is not evaluable (n.e.) or NA, patient will be considered to have experienced hypersensitivity reaction.
[b] unconditional exact confidence interval based on Chan and Zhang

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| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Standard Risk** | | | |
| Number of patients | 24 (100.0%) | 24 (100.0%) | 48 (100.0%) |
| Hypersensitivity reactions | | | |
| Yes | 10 ( 41.7%) | 9 ( 37.5%) | 19 ( 39.6%) |
| No | 12 ( 50.0%) | 12 ( 50.0%) | 24 ( 50.0%) |
| n.e. | 2 ( 8.3%) | 3 ( 12.5%) | 5 ( 10.4%) |
| Difference[a] (rASNase - ASNase medac) | | 0.0% | |
| 95% CI[b] | | [-29.6%; 29.6%] | |
| Allergic reaction within 12 hours after ASNase infusion | | | |
| Yes | 2 ( 8.3%) | 0 (0%) | 2 ( 4.2%) |
| No | 22 ( 91.7%) | 24 (100.0%) | 46 ( 95.8%) |
| Silent inactivation | | | |
| Yes | 10 ( 41.7%) | 9 ( 37.5%) | 19 ( 39.6%) |
| No | 12 ( 50.0%) | 12 ( 50.0%) | 24 ( 50.0%) |
| n.e. | 2 ( 8.3%) | 3 ( 12.5%) | 5 ( 10.4%) |

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Medium Risk** | | | |
| Number of patients | 63 (100.0%) | 60 (100.0%) | 123 (100.0%) |
| Hypersensitivity reactions | | | |
| Yes | 24 ( 38.1%) | 26 ( 43.3%) | 50 ( 40.7%) |
| No | 36 ( 57.1%) | 31 ( 51.7%) | 67 ( 54.5%) |
| n.e. | 3 ( 4.8%) | 3 ( 5.0%) | 6 ( 4.9%) |
| Difference[a] (rASNase - ASNase medac) | | 5.5% | |
| 95% CI[b] | | [-12.6%; 23.06%] | |
| Allergic reaction within 12 hours after ASNase infusion | | | |
| Yes | 0 (0%) | 5 ( 8.3%) | 5 ( 4.1%) |
| No | 63 (100.0%) | 55 ( 91.7%) | 118 ( 95.9%) |
| Silent inactivation | | | |
| Yes | 24 ( 38.1%) | 22 ( 36.7%) | 46 ( 37.4%) |
| No | 36 ( 57.1%) | 31 ( 51.7%) | 67 ( 54.5%) |
| n.e. | 3 ( 4.8%) | 7 ( 11.7%) | 10 ( 8.1%) |

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **High Risk** | | | |
| Number of patients | 8 ( 100%) | 8 ( 100%) | 16 ( 100%) |
| Hypersensitivity reactions | | | |
| Yes | 5 ( 63%) | 2 ( 25%) | 7 ( 44%) |
| No | 3 ( 38%) | 6 ( 75%) | 9 ( 56%) |
| Difference[a] (rASNase - ASNase medac) | | -37.5% | |
| 95% CI[b] | | [-78.0%; 17.3%] | |
| Allergic reaction within 12 hours after ASNase infusion | | | |
| Yes | 5 ( 63%) | 1 ( 13%) | 6 ( 38%) |
| No | 3 ( 38%) | 7 ( 88%) | 10 ( 63%) |
| Silent inactivation | | | |
| Yes | 2 ( 25%) | 1 ( 13%) | 3 ( 19%) |
| No | 3 ( 38%) | 6 ( 75%) | 9 ( 56%) |
| n.e. | 3 ( 38%) | 1 ( 13%) | 4 ( 25%) |

Source: Listing 16.2.7A, Listing 16.2.7B, Listing 16.2.7C, Listing 16.2.7D, Listing 16.2.7E, Listing 16.2.7F
[a] If hypersensitivity reaction is not evaluable (n.e.) or NA, patient will be considered to have experienced hypersensitivity reaction.
[b] unconditional exact confidence interval based on Chan and Zhang

In the asparaginase medac group the allergic reactions were of CTCAE Grade II in one patient and of CTCAE Grades III in six patients. In the recombinant asparaginase group the allergic reactions were of CTCAE Grades I, II and IV in one patient each and of CTCAE Grade III in three patients.

**Table 52: Cumulative frequency of patients with anti-ASNase antibodies in serum during specific treatment phases – overall and stratified by risk group (SAS) (Study MC-ASP.5/ALL)**

| | ASNase medac | rASNase | Total |
|---|---|---|---|
| **Overall** | | | |
| Anti-ASNase antibodies prior to first infusion in induction phase | | | |
| Number of patients | 101 (100.0%) | 97 (100.0%) | 198 (100.0%) |
| Positive | 4 ( 4.0%) | 1 ( 1.0%) | 5 ( 2.5%) |
| Negative | 94 ( 93.1%) | 93 ( 95.9%) | 187 ( 94.4%) |
| NK | 3 ( 3.0%) | 3 ( 3.1%) | 6 ( 3.0%) |
| Difference[a] (rASNase - ASNase medac) | -2.8% | | |
| 95% CI[b] | [-10.13%; 4.09%] | | |
| Anti-ASNase antibodies until start of post-induction phase | | | |
| Positive | 53 ( 52.5%) | 53 ( 54.6%) | 106 ( 53.5%) |
| Negative | 48 ( 47.5%) | 43 ( 44.3%) | 91 ( 46.0%) |
| NK | 0 (0%) | 1 ( 1.0%) | 1 ( 0.5%) |
| Difference[a] (rASNase - ASNase medac) | 3.2% | | |
| 95% CI[b] | [-10.81%; 17.24%] | | |
| Anti-ASNase antibodies until end of post-induction phase | | | |
| Positive | 55 ( 54.5%) | 58 ( 59.8%) | 113 ( 57.1%) |
| Negative | 46 ( 45.5%) | 38 ( 39.2%) | 84 ( 42.4%) |
| NK | 0 (0%) | 1 ( 1.0%) | 1 ( 0.5%) |
| Difference[a] (rASNase - ASNase medac) | 6.4% | | |
| 95% CI[b] | [-7.52%; 20.43%] | | |

Source: Listing 16.2.6A, Listing 16.2.6C, Listing 16.2.6D, Listing 16.2.6E
[a] If Anti-ASNase antibodies are NK, patient will be considered to have Anti-ASNase antibodies.
[b] unconditional exact confidence interval based on Chan and Zhang

Thirty six (36) patients of the 37 hypersensitive patients in the Asparaginase medac group tested positive for anti-asparaginase antibodies. Of these 35 were already antibody-positive before administration of first asparaginase dose during the post-induction phase. Of the 36 hypersensitive patients in the recombinant asparaginase group, 33 had anti-drug antibodies. 30 were already antibody-positive before administration of the first asparaginase dose during post-induction phase. 16 patients in the Asparaginase medac group and 22 patients in the recombinant asparaginase group were positive for anti-asparaginase antibodies before and/or after first asparaginase dose in post-induction treatment, but did not show signs of clinical allergy or silent inactivation. Of these patients, 10 patients in the Asparaginase medac group and 12 patients in the recombinant asparaginase group could fulfil their full course of asparaginase treatment, but six patients in the Asparaginase medac group and 10 patients in the recombinant asparaginase group switched to an alternative asparaginase later.

*Adverse Events during the Induction Phase*

**Table 53: Adverse Events Occurring in at least 5% of Patients in one of the Treatment Groups during Induction Phase - Safety Analysis Set (Study MC-ASP.5/ALL)**

| CTCAE Category | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Number of patients | 101 | (100.0%) | 97 | (100.0%) | 198 | (100.0%) |
| Patients with any event | 97 | ( 96.0%) | 91 | ( 93.8%) | 188 | ( 94.9%) |
| Pain | | | | | | |
| Any event | 51 | ( 50.5%) | 50 | ( 51.5%) | 101 | ( 51.0%) |
| Pain [Abdomen NOS] | 18 | ( 17.8%) | 20 | ( 20.6%) | 38 | ( 19.2%) |
| Pain [Extremity-limb] | 21 | ( 20.8%) | 11 | ( 11.3%) | 32 | ( 16.2%) |
| Pain [Head/headache] | 14 | ( 13.9%) | 17 | ( 17.5%) | 31 | ( 15.7%) |
| Pain [Back] | 8 | ( 7.9%) | 13 | ( 13.4%) | 21 | ( 10.6%) |
| Pain [Bone] | 7 | ( 6.9%) | 11 | ( 11.3%) | 18 | ( 9.1%) |
| Pain [Stomach] | 4 | ( 4.0%) | 8 | ( 8.2%) | 12 | ( 6.1%) |
| Pain [Throat/pharynx/larynx] | 6 | ( 5.9%) | 5 | ( 5.2%) | 11 | ( 5.6%) |
| Gastrointestinal | | | | | | |
| Any event | 48 | ( 47.5%) | 52 | ( 53.6%) | 100 | ( 50.5%) |
| Diarrhea | 19 | ( 18.8%) | 18 | ( 18.6%) | 37 | ( 18.7%) |
| Vomiting | 16 | ( 15.8%) | 12 | ( 12.4%) | 28 | ( 14.1%) |
| Constipation | 13 | ( 12.9%) | 13 | ( 13.4%) | 26 | ( 13.1%) |
| Nausea | 11 | ( 10.9%) | 14 | ( 14.4%) | 25 | ( 12.6%) |
| Mucositis (clinical exam) [Oral cavity] | 10 | ( 9.9%) | 12 | ( 12.4%) | 22 | ( 11.1%) |
| Anorexia | 6 | ( 5.9%) | 3 | ( 3.1%) | 9 | ( 4.5%) |
| Constitutional symptoms | | | | | | |
| Any event | 44 | ( 43.6%) | 53 | ( 54.6%) | 97 | ( 49.0%) |
| Fatigue | 35 | ( 34.7%) | 33 | ( 34.0%) | 68 | ( 34.3%) |
| Insomnia | 5 | ( 5.0%) | 11 | ( 11.3%) | 16 | ( 8.1%) |
| Weight loss | 4 | ( 4.0%) | 9 | ( 9.3%) | 13 | ( 6.6%) |
| Neurology | | | | | | |
| Any event | 45 | ( 44.6%) | 46 | ( 47.4%) | 91 | ( 46.0%) |
| Mood alteration [Agitation] | 13 | ( 12.9%) | 17 | ( 17.5%) | 30 | ( 15.2%) |
| Neuropathy-motor | 16 | ( 15.8%) | 14 | ( 14.4%) | 30 | ( 15.2%) |
| Neuropathy-sensory | 11 | ( 10.9%) | 12 | ( 12.4%) | 23 | ( 11.6%) |
| Dizziness | 1 | ( 1.0%) | 6 | ( 6.2%) | 7 | ( 3.5%) |

continued

| CTCAE Category | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Infection | | | | | | |
| Any event | 25 | ( 24.8%) | 29 | ( 29.9%) | 54 | ( 27.3%) |
| Infection (documented clinically) [Oral cavity-gums (gingivitis)] | 6 | ( 5.9%) | 8 | ( 8.2%) | 14 | ( 7.1%) |
| Febrile neutropenia | 5 | ( 5.0%) | 6 | ( 6.2%) | 11 | ( 5.6%) |
| Infection with normal ANC [Oral cavity-gums (gingivitis)] | 4 | ( 4.0%) | 5 | ( 5.2%) | 9 | ( 4.5%) |
| Dermatology/skin | | | | | | |
| Any event | 22 | ( 21.8%) | 23 | ( 23.7%) | 45 | ( 22.7%) |
| Rash | 9 | ( 8.9%) | 8 | ( 8.2%) | 17 | ( 8.6%) |
| Dermatology - Other | 5 | ( 5.0%) | 7 | ( 7.2%) | 12 | ( 6.1%) |
| Hemorrhage/bleeding | | | | | | |
| Any event | 14 | ( 13.9%) | 13 | ( 13.4%) | 27 | ( 13.6%) |
| Hemorrhage pulmonary [Nose] | 3 | ( 3.0%) | 6 | ( 6.2%) | 9 | ( 4.5%) |
| Musculoskeletal/soft tissue | | | | | | |
| Any event | 14 | ( 13.9%) | 11 | ( 11.3%) | 25 | ( 12.6%) |
| Muscle weakness [Extremity-lower] | 12 | ( 11.9%) | 10 | ( 10.3%) | 22 | ( 11.1%) |
| Pulmonary/upper respiratory | | | | | | |
| Any event | 9 | ( 8.9%) | 10 | ( 10.3%) | 19 | ( 9.6%) |
| Cardiac general | | | | | | |
| Any event | 11 | ( 10.9%) | 7 | ( 7.2%) | 18 | ( 9.1%) |
| Hypertension | 9 | ( 8.9%) | 7 | ( 7.2%) | 16 | ( 8.1%) |
| Vascular | | | | | | |
| Any event | 6 | ( 5.9%) | 7 | ( 7.2%) | 13 | ( 6.6%) |
| Vascular - Other | 4 | ( 4.0%) | 5 | ( 5.2%) | 9 | ( 4.5%) |
| Endocrine | | | | | | |
| Any event | 7 | ( 6.9%) | 1 | ( 1.0%) | 8 | ( 4.0%) |
| Hepatobiliary/pancreas | | | | | | |
| Any event | 6 | ( 5.9%) | 1 | ( 1.0%) | 7 | ( 3.5%) |

Source: Table 14.3.1.2A, Listing 16.2.7H, Listing 16.2.7I

During induction phase, AEs occurred in 97 patients (96.0%) receiving asparaginase medac and in 91 patients (93.8%) receiving recombinant asparaginase. Asparaginase-typical AEs were observed in 46 patients (47.4%) receiving recombinant asparaginase and in 51 patients (50.5%) receiving asparaginase medac.

Allergic reactions within 12 hours after asparaginase infusion were observed in five patients (5.0%) receiving asparaginase medac. These were of CTCAE Grades I and III in one patient each and of CTCAE Grade II in three patients. The allergic reactions within 12 hours after asparaginase infusion that were observed in two patients (2.1%) receiving recombinant asparaginase were of CTCAE Grades I and II in one patient each.

**Table 54: Frequency of patients with allergic reactions (MC-ASP.5/ALL; Safety analysis set)**

| **Treatment group** | **Spectrila** | **Reference asparaginase** |
|---|---|---|
| Number of patients | 97 | 101 |
| Allergic reactions within 12 hours after asparaginase infusion during induction treatment | 2 (2.1%) | 5 (5.0%) |
| Any allergic event* within 24 hours after asparaginase infusion during induction treatment | 16 (16%) | 24 (24%) |
| | | |
| **Including all allergic reactions within 12 hours after asparaginase infusion and all adverse events with CTCAE terms syncope (fainting), hypotension, rash, flushing, pruritus, dyspnoea, injection site reaction or airway obstruction within 24 hours after asparaginase infusion* | | |

Drug-related AEs occurring in at least 5% of patients during induction phase are presented below.

**Table 55: Drug-Related Adverse Events occurring in at least 5% of Patients in One Treatment Group during Induction Phase - Safety Analysis Set (Study MC-ASP.5/ALL)**

| CTCAE Category | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Number of patients | 101 | (100.0%) | 97 | (100.0%) | 198 | (100.0%) |
| Patients with any event | 33 | ( 32.7%) | 36 | ( 37.1%) | 69 | ( 34.8%) |
| Infection | | | | | | |
| Any event | 6 | ( 5.9%) | 12 | ( 12.4%) | 18 | ( 9.1%) |
| Gastrointestinal | | | | | | |
| Any event | 7 | ( 6.9%) | 9 | ( 9.3%) | 16 | ( 8.1%) |
| Nausea | 3 | ( 3.0%) | 7 | ( 7.2%) | 10 | ( 5.1%) |
| Constitutional symptoms | | | | | | |
| Any event | 6 | ( 5.9%) | 7 | ( 7.2%) | 13 | ( 6.6%) |
| Fatigue | 6 | ( 5.9%) | 5 | ( 5.2%) | 11 | ( 5.6%) |
| Vascular | | | | | | |
| Any event | 6 | ( 5.9%) | 7 | ( 7.2%) | 13 | ( 6.6%) |
| Vascular - Other | 4 | ( 4.0%) | 5 | ( 5.2%) | 9 | ( 4.5%) |
| Hepatobiliary/pancreas | | | | | | |
| Any event | 6 | ( 5.9%) | 1 | ( 1.0%) | 7 | ( 3.5%) |
| Hemorrhage/bleeding | | | | | | |
| Any event | 1 | ( 1.0%) | 5 | ( 5.2%) | 6 | ( 3.0%) |

Source: Table 14.3.1.2E, Listing 16.2.7H, Listing 16.2.7I

*Adverse Events during Post-Induction Phase for High Risk Patients*

In the asparaginase medac group infection was observed in four patients (50%). Constitutional symptoms, gastrointestinal events, pain and dermatology/skin events were each observed in three patients (38%). In the recombinant asparaginase group infection was observed in six patients (75%),

constitutional symptoms were observed in five patients (63%), gastrointestinal events and pain were each observed in four patients (50%) and haemorrhage/bleeding was observed in three patients (38%).

**Table 56: High Risk Patients with Asparaginase-Typical AEs during Post-Induction Treatment - Safety Analysis Set (Study MC-ASP.5/ALL)**

| ASNase-typical adverse events | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Number of patients | 8 | (100%) | 8 | (100%) | 16 | (100%) |
| Patients with any ASNase-typical adverse events | 8 | (100%) | 3 | ( 38%) | 11 | ( 69%) |
| Difference in Rates [%] (rASNase-ASNase medac) | | | -63% | | | |
| 95% CI[a] | | | [-91.5%; -17.3%] | | | |
| Allergic reaction within 12 hours after ASNase infusion | 6 | ( 75%) | 2 | ( 25%) | 8 | ( 50%) |
| Liver enzymes ( ≥CTCAE Grade III) | | | | | | |
| Any event | 1 | ( 13%) | 1 | ( 13%) | 2 | ( 13%) |
| ALT/GPT | 1 | ( 13%) | 1 | ( 13%) | 2 | ( 13%) |
| Hyperbilirubinemia | 0 | (0%) | 1 | ( 13%) | 1 | ( 6%) |
| Haemorrhage or thromboembolism ( ≥CTCAE Grade II) | | | | | | |
| Any event | 2 | ( 25%) | 0 | (0%) | 2 | ( 13%) |
| Hemorrhage, GI [Oral cavity] | 1 | ( 13%) | 0 | (0%) | 1 | ( 6%) |
| Petechiae | 1 | ( 13%) | 0 | (0%) | 1 | ( 6%) |

Source: Listing 16.2.7J, Listing 16.2.7K, Listing 16.2.8D
[a]unconditional exact confidence interval based on Chan and Zhang

- Study - MC-ASP.4/ALL

During induction phase A, 69 events occurred in 14 subjects (87.5%) under the test and 74 events in 16 subjects (100%) under the reference treatment. One event was observed during induction phase B. Most patients had gastrointestinal adverse events (75%), followed by neurological events (56.3%), pain (40.6%), infections (28.1%), constitutional symptoms (25%), and dermal events (12.5%). The event during induction phase B (post-asparaginase medac) was severe venous thrombosis at a vascular access site. No deaths occurred during the study. Twenty-seven events observed in 15 subjects (46.9%), mainly gastrointestinal events (40.6%), were assessed to be at least possibly related to the study drug.

- Study MC-ASP.2/RHN

In total, 32 adverse events were observed in five patients with three events (pneumonia, septicaemia, and femoral neck fracture) being serious. Only one event (elevation of alkaline phosphatase) was assessed to be related to recombinant L-asparaginase by the investigator; all other events were considered to be unlikely or not related to the study drug. 75% were of mild or moderate intensity (CTC grade 1 or 2). The most common adverse events involved constitutional symptoms, metabolic/laboratory changes, pain, gastrointestinal symptoms, and infections.

- Study MC-ASP.6/ALL

All 12 patients experienced at least one AE. Seven patients (58%) experienced AEs that were at least CTCAE grade III, 3 patients (25%) experienced AEs that were drug related (rash, nausea, febrile neutopaenia, thrombosis). Three patients (25%) experienced at least one AE typical for asparaginase (nose bleed, thrombosis of the superior vena cava superior, elevated level of ALT & GPT).

The primary endpoint of study MC-ASP.6/ALL was hypersensitivity reactions during induction.

**Table 57: Allergic Reactions, Silent Inactivation and Hypersensitivity**

| | n (%) | Exact 90%-Confidence interval* |
|---|---|---|
| Number of patients | 12 (100%) | |
| Patients with allergic reactions[a] | 0 ( 0%) | [ 0%, 22%] |
| Patients with ASNase trough serum activity <20 U/L before ASNase administration number 2, 4 or 6[b] | 4 ( 33%) | [ 12%, 61%] |
| Patients with hypersensitivity reaction[c] | 4 ( 33%) | [ 12%, 61%] |

Source: Listing 16.2.7A
* Pearson-Clopper Confidence interval
a within 12 hours after rASNase infusion
b silent inactivation according to definition in study protocol
c combined endpoint of allergic reaction and silent inactivation

No allergic reactions were observed in any of the 12 infants < 1 year of age during treatment with Spectrila.

In 2 of the 4 patients asparaginase trough levels were below 20 U/L on Day 33, in 1 patient on Day 25 and Day 33 and in 1 patient on Day 25 only. No clinical allergic reaction was documented within 12 hours after IP infusion. No antibodies against asparaginase were detected in any of the patients.

Serum levels of anti-asparaginase antibodies were measured directly before recombinant asparaginase infusions on Days 15 (baseline value), 18, 25 and 33. All patients measured on these days were negative for anti-recombinant asparaginase antibodies.

- Study MC-ASP.1/ALL

The most frequently reported adverse events were gastrointestinal symptoms, which are known to be typical asparaginase side effects, as are the observed alterations in coagulation parameters.

Adverse drug reactions

Adverse events were pooled for the two pivotal trials MC-ASP.4/ALL and MC-ASP.5/ALL and presented using MedDRA terms. For mapping purposes in study MC-ASP.5/ALL CTCAE version 3.0 was firstly mapped to CTCAE version 4, followed by mapping to MedDRA version 12.1. The table showing drug-related AEs and Grade≥3 drug-related AEs in the safety analysis set are reported below:

**Table 58: Frequency of patients with Grade ≥3 and drug-related AEs by SOC and PT during induction phase (MC-ASP.4/ALL-ITT, MC-ASP.5/ALL-Safety Analysis Set)**

| SOC | CTCAE | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|---|
| PT | Grade | n | (%) | n | (%) | n | (%) |
| **Number of patients** | | 117 | (100.0%) | 113 | (100.0%) | 230 | (100.0%) |
| **Patients with any event** | | 15 | ( 12.8%) | 20 | ( 17.7%) | 35 | ( 15.2%) |
| **Nervous system disorders** | | | | | | | |
| Any event | Any | 4 | ( 3.4%) | 5 | ( 4.4%) | 9 | ( 3.9%) |
| Intracranial venous sinus thrombosis | Any | 1 | ( 0.9%) | 3 | ( 2.7%) | 4 | ( 1.7%) |
| | III | 0 | (0%) | 2 | ( 1.8%) | 2 | ( 0.9%) |
| | IV | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| Cerebral venous thrombosis | Any | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| Convulsion | Any | 2 | ( 1.7%) | 0 | (0%) | 2 | ( 0.9%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Superior sagittal sinus thrombosis | Any | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| | III | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| Haemorrhage intracranial | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Headache | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| **Infections and infestations** | | | | | | | |
| Any event | Any | 2 | ( 1.7%) | 5 | ( 4.4%) | 7 | ( 3.0%) |
| Opportunistic infection | Any | 0 | (0%) | 2 | ( 1.8%) | 2 | ( 0.9%) |
| | III | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | V | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| Sepsis | Any | 2 | ( 1.7%) | 0 | (0%) | 2 | ( 0.9%) |
| | III | 2 | ( 1.7%) | 0 | (0%) | 2 | ( 0.9%) |
| Encephalitic infection | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | V | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| Gingival infection | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | III | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |

| SOC<br>PT | CTCAE<br>Grade | ASNase medac<br>n | <br>(%) | rASNase<br>n | <br>(%) | Total<br>n | <br>(%) |
|---|---|---|---|---|---|---|---|
| Hepatic infection | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | III | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| **Blood and lymphatic system disorders** | | | | | | | |
| Any event | Any | 2 | ( 1.7%) | 4 | ( 3.5%) | 6 | ( 2.6%) |
| Febrile neutropenia | Any | 2 | ( 1.7%) | 4 | ( 3.5%) | 6 | ( 2.6%) |
| | III | 2 | ( 1.7%) | 4 | ( 3.5%) | 6 | ( 2.6%) |
| **Metabolism and nutrition disorders** | | | | | | | |
| Any event | Any | 3 | ( 2.6%) | 3 | ( 2.7%) | 6 | ( 2.6%) |
| Hypertriglyceridaemia | Any | 1 | ( 0.9%) | 2 | ( 1.8%) | 3 | ( 1.3%) |
| | IV | 1 | ( 0.9%) | 2 | ( 1.8%) | 3 | ( 1.3%) |
| Diabetes mellitus | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Glucose tolerance impaired | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Metabolic syndrome | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | V | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| **Vascular disorders** | | | | | | | |
| Any event | Any | 3 | ( 2.6%) | 3 | ( 2.7%) | 6 | ( 2.6%) |
| Embolism | Any | 2 | ( 1.7%) | 2 | ( 1.8%) | 4 | ( 1.7%) |
| | III | 2 | ( 1.7%) | 1 | ( 0.9%) | 3 | ( 1.3%) |
| | IV | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| Thrombosis | Any | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| | III | 1 | ( 0.9%) | 1 | ( 0.9%) | 2 | ( 0.9%) |
| **Investigations** | | | | | | | |
| Any event | Any | 0 | (0%) | 4 | ( 3.5%) | 4 | ( 1.7%) |
| Blood cholesterol increased | Any | 0 | (0%) | 2 | ( 1.8%) | 2 | ( 0.9%) |
| | IV | 0 | (0%) | 2 | ( 1.8%) | 2 | ( 0.9%) |
| Activated partial thromboplastin time | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | III | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| Gamma-glutamyltransferase increased | Any | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |
| | IV | 0 | (0%) | 1 | ( 0.9%) | 1 | ( 0.4%) |

| SOC<br>PT | CTCAE<br>Grade | ASNase medac<br>n | <br>(%) | rASNase<br>n | <br>(%) | Total<br>n | <br>(%) |
|---|---|---|---|---|---|---|---|
| **Hepatobiliary disorders** | | | | | | | |
| Any event | Any | 3 | ( 2.6%) | 0 | (0%) | 3 | ( 1.3%) |
| Hepatic failure | Any | 2 | ( 1.7%) | 0 | (0%) | 2 | ( 0.9%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Cholestasis | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| **Gastrointestinal disorders** | | | | | | | |
| Any event | Any | 2 | ( 1.7%) | 0 | (0%) | 2 | ( 0.9%) |
| Nausea | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Pancreatitis | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | IV | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Vomiting | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| **Respiratory, thoracic and mediastinal disorders** | | | | | | | |
| Any event | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| Pleural effusion | Any | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |
| | III | 1 | ( 0.9%) | 0 | (0%) | 1 | ( 0.4%) |

Additional ADRs were listed in the proposed SmPC of Spectrila which were not observed in the clinical development programme but are well-known undesired effects of ASNase treatment. For frequency

assignment, the frequencies observed in clinical trials and frequencies reported with all other ASNase preparations have been considered.

Based on the above the following ADRs have been included in the SmPC:

| System organ class | Frequency and symptom |
|---|---|
| Infections and infestations | **Not known**<br>Infections |
| Blood and lymphatic system disorders | **Common**<br>Disseminated intravascular coagulation (DIC), anaemia, leukopenia, thrombocytopenia |
| Immune system disorders | **Very common**<br>Hypersensitivity including flushing, rash, hypotension, oedema/angioedema, urticaria, dyspnoea<br><br>**Common**<br>Hypersensitivity including bronchospasm<br><br>**Rare**<br>Anaphylactic shock |
| Endocrine disorders | **Very rare**<br>Secondary hypothyroidism, hypoparathyroidism |
| Metabolism and nutrition disorders | **Very common**<br>Hyperglycaemia, hypoalbuminaemia<br><br>**Common**<br>Hypoglycaemia, decreased appetite, weight loss<br><br>**Uncommon**<br>Hyperuricaemia, hyperammonaemia<br><br>**Rare**<br>Diabetic ketoacidosis |
| Psychiatric disorders | **Common**<br>Depression, hallucination, confusion |
| Nervous system disorders | **Common**<br>Neurological signs and symptoms including agitation, dizziness and somnolence<br><br>**Uncommon**<br>Headaches<br><br>**Rare**<br>Ischaemic stroke, reversible posterior leukoencephalopathy syndrome (RPLS), convulsion, disturbances in consciousness including coma<br><br>**Very rare**<br>Tremor |
| Vascular disorders | **Common**<br>Thrombosis especially cavernous sinus thrombosis or deep vein thrombosis, haemorrhage |
| Gastrointestinal disorders | **Very common**<br>Diarrhoea, nausea, vomiting, abdominal pain<br><br>**Common**<br>Acute pancreatitis<br><br>**Rare**<br>Haemorrhagic pancreatitis, necrotising pancreatitis, parotitis<br><br>**Very rare**<br>Pancreatitis with fatal outcome, pancreatic pseudocyst |

| Hepatobiliary disorders | **Rare**<br>Hepatic failure with potentially fatal outcome, hepatic necrosis, cholestasis, jaundice<br><br>**Not known**<br>Hepatic steatosis |
|---|---|
| General disorders and administration site conditions | **Very common**<br>Oedema, fatigue<br><br>**Common**<br>Pain (back pain, joint pain) |
| Investigations | **Very common**<br>Increase in transaminases, blood bilirubin, blood alkaline phosphatase, blood cholesterol, blood triglyceride, very low density lipoprotein (VLDL), lipoprotein lipase activity, blood urea, ammonia, blood lactate dehydrogenase (LDH),<br>Decrease in antithrombin III, blood fibrinogen, blood cholesterol, low density lipoprotein (LDL), total protein<br><br>**Common**<br>Increase in amylase, lipase, abnormal electroencephalogram (EEG) (reduced alpha wave activity, increased theta and delta wave activity) |

***Serious adverse event/deaths/other significant events***

- *Study MC-ASP.5/ALL*

*Serious Adverse Events during Induction Phase*

**Table 59: SAEs occurring in at least 5% of Patients in one of the Treatment Groups during Induction Phase - Safety Analysis Set (Study MC-ASP.5/ALL)**

| CTCAE Category | ASNase medac | | rASNase | | Total | |
|---|---|---|---|---|---|---|
| CTCAE Term | n | (%) | n | (%) | n | (%) |
| Number of patients | 101 | (100.0%) | 97 | (100.0%) | 198 | (100.0%) |
| Patients with any event | 35 | ( 34.7%) | 28 | ( 28.9%) | 63 | ( 31.8%) |
| Infection | | | | | | |
| Any event | 12 | ( 11.9%) | 10 | ( 10.3%) | 22 | ( 11.1%) |
| Febrile neutropenia | 5 | ( 5.0%) | 6 | ( 6.2%) | 11 | ( 5.6%) |
| Gastrointestinal | | | | | | |
| Any event | 11 | ( 10.9%) | 5 | ( 5.2%) | 16 | ( 8.1%) |
| Vascular | | | | | | |
| Any event | 5 | ( 5.0%) | 7 | ( 7.2%) | 12 | ( 6.1%) |
| Vascular - Other | 4 | ( 4.0%) | 5 | ( 5.2%) | 9 | ( 4.5%) |

Source: Table 14.3.2A, Listing 16.2.7H, Listing 16.2.7I

*Serious Adverse Events during Post-Induction Phase – High Risk Patients Only*

During post-induction phase, SAEs were observed in three HR patients (38%) receiving asparaginase medac and in six HR patients (75%) receiving recombinant asparaginase. Infections were the most frequent AEs in both arms, with all patients suffering SAEs in the recombinant asparaginase arm also suffering a serious infection.

*Deaths*

A total of four deaths occurred during the course of this clinical study.

**Table 60: Display of Deaths Listed By Patient - Safety Analysis Set (Study MC-ASP.5/ALL)**

| Treatment/ Pat ID | Patient's age at random-isation [years] | First study drug infusion during induction phase [yyyy-mm-dd] | Termination of study participation during | Date of death [yyyy-mm-dd] | Reason of death |
|---|---|---|---|---|---|
| ASNase medac | | | | | |
| | 2 | 2010-02-22 | Intensification phase | 2011-07-28 | Intracerebral Aspergillus infection |
| rASNase | | | | | |
| | 7 | 2009-12-16 | Induction phase | 2009-12-21 | Sudden death |
| | 9 | 2010-11-30 | Induction phase | 2010-12-27 | Opportunistic infection |
| | 12 | 2011-05-31 | Induction phase | 2011-06-25 | Aspergillus infection |

Source: Listing 16.2.1A, Listing 16.2.6I, Listing 16.2.6J

- *Study - MC-ASP.4/ALL*

In three patients receiving Asparaginase medac, treatment led to a temporary discontinuation of study drug in two and a permanent discontinuation in one.

**Table 61: Severe or life-threatening adverse events - ITT population (Study MC-ASP.4/ALL)**

| CTC category | Recombinant ASNase (MC1003, test) N=16 | Asparaginase medac™ (MC0904, reference) N=16 | Total N=32 |
|---|---|---|---|
| **Induction phase A** | | | |
| Infection | 4 (3) 18.8% | 12 (6) 37.5% | 16 (9) 28.1% |
| Neurology | 2 (2) 12.5% | 4 (3) 18.8% | 6 (5) 15.6% |
| Gastrointestinal | 3 (3) 18.8% | 1 (1) 6.3% | 4 (4) 12.5% |
| Pain | - | 1 (1) 6.3% | 1 (1) 3.1% |
| Constitutional symptoms | 1 (1) 6.3% | 1 (1) 6.3% | 2 (2) 6.6% |
| Endocrine | 1 (1) 6.3% | - | 1 (1) 3.1% |
| Metabolic/Laboratory | 1 (1) 6.3% | - | 1 (1) 3.1% |
| Vascular | 1 (1) 6.3% | - | 1 (1) 3.1% |
| **Total** | **13 (7) 43.8%** | **19 (8) 50%** | **32 (15) 46.9%** |
| **Induction phase B** | | | |
| Vascular | - | 1 (1) 6.3% | 1 (1) 3.1% |
| **Total** | - | **1 (1) 6.3%** | **1 (1) 3.1%** |

Source: Table 14.3.1.4
f= number of events, n= number of patients, %= percentage of patients treated

- Study MC-ASP.2/RHN

No deaths occurred during the study but one occurred shortly after. Two patients had SAEs: pneumonia (1) and fracture and septicaemia causing death (1).

- Study MC-ASP.6/ALL

No patient died during the course of this clinical study. Four patients (33%) experienced at least one SAE during the course of this study (Infection (17%), pulmonary/upper respiratory event (8%), vascular event (8%)).

- Study MC-ASP.1/ALL

No serious or other significant adverse event led to temporary or permanent discontinuation of the study drug. No deaths occurred in the study.

***Laboratory findings***

- *Study MC-ASP.5/ALL*

*Serum Chemistry during Induction Phase*

The median creatinine levels remained steady and unchanged during the induction phase. Bilirubin levels were elevated to between approximately 1.0- and 0.61-fold (upper limit of normal) ULN from 0.590-fold ULN at baseline. Median AST and ALT estimates dipped by approximately a third during the study. Serum amylase levels were raised slightly in the early part of the induction phase from a baseline of 0.580-fold ULN. However levels dropped to approximately 85% of baseline levels toward the end of the study. Glucose levels also dropped from a baseline of approximately 6 mmol/L to between 4 and 5 mmol/L. The median baseline levels of total protein were 64 g/L in patients receiving asparaginase medac and 66 g/L in patients receiving recombinant asparaginase. During induction phase median levels were between 58 g/L and 48 g/L in the asparaginase medac treatment group and between 59 g/L and 48 g/L in the recombinant asparaginase treatment group.  There was no difference in the pattern of changes described above between the study arms.

*Coagulation during Induction Phase*

For the majority of the induction period fibrinogen levels dipped to approximately 50% of baseline levels. However, during the 6th week of the induction period, levels rose rapidly to approximately 2.5-fold LLN. Similarly antithrombin III levels fell by approximately 50% from baseline until week 6 where levels rose to just under baseline values. Just as with the serum biochemistry data changes in the asparaginase medac group were mirrored in the recombinant asparaginase group.

*Haematology during Induction Phase*

For leukocytes, levels fell to <50% of baseline, rising to above baseline levels by the $5^{th}$ week.  The median baseline levels of leukocytes were 1.60 g/L in both treatment groups. During induction median levels were between 1.76 g/L and 0.72 g/L in the asparaginase medac group and between 1.75 g/L and 0.88 g/L in the recombinant asparaginase group. Thrombocytes steadily rose from baseline (approximately 62g/L) to a peak of 150 g/L in the asparaginase medac group and 154 g/L in the recombinant asparaginase group.

- *Study - MC-ASP.4/ALL*

One patient had low sodium concentrations in serum (CTC v.3.0, grade 3=severe) on Day 33, all other abnormal values were mild or moderate. No differences over time in safety laboratory values between the two treatments were observed.

- Study MC-ASP.2/RHN

Deviations from the normal values (CTC grade 3, rarely 4) were observed in a few blood chemistry parameters, most coagulation parameters, and in the haemogram. CTC grade 3/4 changes in liver parameters (i.e. elevation of gamma-glutamyltransferase, γ-GT) and a decrease in total serum protein concentration as a consequence of impaired protein synthesis, were observed in two patients and are well-known and labelled side effects of treatment with L-asparaginase. Disturbances in the coagulation system are also known typical side effects. All seven patients showed pronounced changes in coagulation parameters.

- Study MC-ASP.6/ALL

Median safety laboratory parameters showed little variability. Low levels in blood cell counts were attributed to the underlying disease and treatment. Coagulation parameters decreased slightly during the treatment phase which could be attributed to treatment with recombinant asparaginase.

***Immunogenicity***

In the study in children/adolescents aged 1 – 18 years with de novo ALL (MC-ASP.5/ALL), by day 33 of induction treatment 10 patients in the Spectrila group (10.3 %) and 9 in the reference group (8.9 %) were measured positive for anti-asparaginase antibodies at least at one time point.

A comparable proportion of patients in both groups developed anti-asparaginase antibodies before the start of the post-induction treatment phase (Spectrila 54.6 % vs. reference E. coli asparaginase 52.5 %). The majority of anti-asparaginase antibodies developed in the time gap between the last L asparaginase infusion on day 33 and start of post-induction treatment at day 79.

No anti-asparaginase antibodies were detected in any of the 12 infants < 1 year of age during treatment with L asparaginase (MC-ASP.6/INF) (see SmPC section 4.8).

### 2.6.1. Discussion on clinical safety

The provided safety database for recombinant asparaginase is limited (data on 135 patients included) which is acceptable considering the rarity of ALL. In the single arm studies, all the planned infusions were administered, with the exception of study MC-ASP.2/RHN enrolling heavily pre-treated or refractory patients in which the full course of treatment was not completed.

Since Spectrila is usually used in combination therapy with other antineoplastic agents, the demarcation from undesirable effects of other medicinal products is often difficult (see SmPC section 4.8).

The profile of adverse effects of recombinant asparaginase was broadly similar to that of asparaginase medac. No noteworthy differences were indicated for events of thrombosis, pancreatitis and liver function changes.

A higher rate of patients with drug-related AEs Grade ≥ III (19.6% vs. 13.9%) and drug-related serious AEs (18.6% vs. 15.8%), was recorded in the recombinant asparaginase arm in the pivotal study compared to asparaginase medac. No further safety signal in addition to those already known to be associated to asparaginase treatment have been registered, even though a slightly increased rate of ADR has been registered with rASNase (37.1% vs. 32.7%), in particular for the terms: Infections (12.4% vs. 5.9%), Gastrointestinal events (9.3% vs. 6.9%), and Haemorrhage/bleeding (5.2% vs. 1.0%). The differences were not however considered clinically relevant and were not generally seen in the other clinical studies.

The primary toxicity of asparaginase results from immunologic reactions caused by exposure to the bacterial protein. Hypersensitivity reactions range from transient flushing or rash and urticaria to bronchospasm, angioedema and anaphylaxis.

Spectrila can induce antibodies of different immunoglobulin classes (IgG, IgM, IgE). These antibodies may induce clinical allergic reactions, inactivate the enzymatic activity or accelerate the elimination of asparaginase. Allergic reactions can manifest as flushing, rash, pain (joint pain, back pain and abdominal pain), hypotension, oedema/angioedema, urticaria, dyspnoea, bronchospasm up to anaphylactic shock. The probability of the occurrence of allergic reactions increases with the number of administered doses; however, in very rare cases reactions can occur at the first dose of asparaginase. Most hypersensitivity reactions to asparaginase are observed during subsequent treatment phases (re-induction treatment, delayed intensification).

Spectrila was not considered to have a more favourable safety profile to that of asparaginase medac, particularly with regard to anti-drug antibody (ADA) development, as was hypothesized by the applicant on the basis of the lower aggregate content of the test product.

The binding antibody data presented indicate a low and similar rate of ADA formation during the induction phase for both study arms. The ADA titres were similar and overall there was no indication of stronger

ADA responses to recombinant asparaginase than to asparaginase medac by incidence or concentration of ADAs. Interestingly, large increases in ADA incidence and titre occurred in both study arms before the first dose of post-induction treatment and then further increases in titre before HR/MR/SR 2 blocks (2nd post-induction phase). Incidence of hypersensitivity reactions also markedly increased, as did incidence of asparaginase inactivation. By the end of the asparaginase induction treatment approximately 50% of patients had developed antibodies. Asparaginase was not administered between the induction and high-risk (HR) block phases. Immune system reconstitution could be a reason for this observation since treatment with corticosteroids and some anti-neoplastic agents had ceased, although this has not been confirmed.  Almost all patients experiencing hypersensitivity reactions were ADA positive, providing evidence of a good correlation between the two. In infant patients (study MC-ASP.6/INF) no ADAs were detected and the possible explanation for the silent inactivation reported in 33% of patients remains unknown.

The anti-drug antibody assay was reasonably well performed. The ADA data refer to binding antibodies. It cannot be excluded that the ADA assay underestimates the incidence and titre of ADAs due to interference with ADA determination by asparaginase. Decreased asparaginase activity has been included as an important identified risk in the RMP.

Overall, the immunogenicity and hypersentivity reactions have been well addressed in the SmPC and RMP. Because of the risk of severe anaphylactic reactions asparaginase should not be administered as a bolus intravenous injection. A previous intracutaneous test or a small intravenous test dose can be used. Both procedures, however, do not allow for predicting accurately which patients will experience an allergic reaction. If allergic symptoms occur, administration of asparaginase must be discontinued immediately and appropriate treatment given, which may include antihistamines and corticosteroids (see section 4.4). Hypersensitivity to the active substance, any native (non-pegylated) E. coli-asparaginase  preparation or to any of the excipients listed in section 6.1 is a contraindication (see SmPC section 4.3).

In addition, measurement of the asparaginase activity level in serum or plasma may be undertaken in order to rule out accelerated reduction of asparaginase activity.  Preferably, levels should be measured three days after the last asparaginase administration, i.e. usually directly before the next dose of asparaginase is given. Low asparaginase activity levels are often accompanied by the appearance of anti-asparaginase antibodies. In such cases, a switch to a different asparaginase preparation should be considered. Expert advice should first be sought (see SmPC section 4.4).

Treatment with asparaginase can also result in disturbances in organ systems which exhibit a high level of protein synthesis. Decreased protein synthesis can predominantly lead to liver impairment, acute pancreatitis, decreased insulin production with hyperglycaemia, decreased production of clotting factors (especially fibrinogen and antithrombin III) leading to coagulation disorders (thrombosis, bleeding), and decreased production of lipoproteins resulting in hypertriglyceridaemia.

In rare cases severe liver impairment has been described, including cholestasis, icterus, hepatic necrosis and hepatic failure with fatal outcome (see sections 4.8 and 4.5). Liver parameters should be monitored closely before and during treatment with asparaginase. Treatment with asparaginase should be interrupted if patients develop severe hepatic impairment (bilirubin > 3 times the upper limit of normal [ULN]; transaminases > 10 times ULN), severe hypertriglyceridaemia, hyperglycaemia or coagulation disorder (e.g. sinus vein thrombosis, severe bleeding). (see sections 4.4 and 4.8). Severe hepatic impairment (bilirubin > 3 times upper limit of normal [ULN]; transaminases > 10 times ULN) is a contraindication (see SmPC section 4.3).

The serum protein level (especially albumin) decreases very commonly in patients treated with asparaginase as a result of impaired protein synthesis (see section 4.4). As a consequence of hypoalbuminaemia, oedema can occur (see SmPC section 4.8). Since serum protein is important for the

binding and transport function of some active substances, the serum protein level should be monitored regularly (see SmPC section 4.4).

Changes in endocrine pancreatic function are observed very commonly during treatment with asparaginase and manifest predominantly as hyperglycaemia. These events are usually transient. Additionally asparaginase may decrease insulin secretion from pancreatic β-cells and impair insulin receptor function. The syndrome is generally self-limiting. However, in rare cases it can result in diabetic ketoacidosis. Concomitant treatment with corticosteroids contributes to this effect. Serum and urine glucose levels should be regularly monitored and managed as clinically indicated (see SmPC sections 4.4 and 4.8). Hypoglycaemia mostly without clinical symptoms has been commonly observed in patients treated with asparaginase. The mechanism leading to this reaction is unknown. (see SmPC section 4.8).

Treatment with asparaginase should be discontinued in patients developing acute pancreatitis. Acute pancreatitis has developed in less than 10 % of patients. A few cases of asparaginase-induced parotitis have been reported in the literature. In rare cases, haemorrhagic or necrotising pancreatitis occurs. There have been isolated reports of fatal outcomes. Clinical symptoms include abdominal pain, nausea, vomiting and anorexia. Serum amylase and lipase are usually elevated, although in some patients they can be normal due to impaired protein synthesis. Patients with severe hypertriglyceridaemia are at increased risk of developing acute pancreatitis. These patients should no longer be treated with any asparaginase preparation (see SmPC sections 4.3, 4.4 and 4.8). Pancreatitis and history of pancreatitis, with prior asparaginase therapy are contraindications (see SmPC section 4.3).

Mild to moderate changes in blood lipid values (e.g. increased or decreased cholesterol, increased triglyceride, increased VLDL fraction and decreased LDL, increased lipoprotein lipase activity) are very commonly observed in patients treated with asparaginase, which in most cases present without clinical symptoms. Concomitant administration of glucocorticoids may be a contributing factor. However, in rare cases severe hypertriglyceridaemia (triglycerides > 1,000 mg/dl) has been reported. Asparaginase-associated hyperlipidaemia should be treated depending on its severity and on clinical symptoms (see SmPC section 4.4).

Due to the inhibition of protein synthesis (decreased synthesis of factors II, V, VII, VIII, and IX, proteins C and S, antithrombin III [AT III]) caused by asparaginase, coagulation disorders can occur which can manifest either as thrombosis, disseminated intravascular coagulation (DIC), or bleeding. The risk of thrombosis seems to be higher than the risk of bleeding. Symptomatic thromboses related to the use of central venous catheters have been described, too. Approximately half of the thrombotic events is localised in cerebral vessels. Sinus vein thrombosis can occur. Ischaemic strokes are rare. Acquired or genetically decreased physiologic coagulation inhibitors (protein C, protein S, antithrombin) are also described in relation to vascular complications. Frequent evaluation of coagulation parameters is important before and during asparaginase treatment. Expert advice should be sought in cases where AT III is decreased (see SmPC section 4.4). Pre-existing known coagulopathy (e.g. haemophilia) and history of serious haemorrhage or serious thrombosis with prior asparaginase therapy are contraindications (see SmPC section 4.3).

In the pivotal study, grade IV thrombocytopenia events (19.6% vs. 10.9%) were more frequently reported in the recombinant asparaginase arm versus asparaginase medac. It cannot be concluded that this is associated with the slight increase in bleeding ADRs seen in this treatment arm as the difference in bleeding rate between the study arms was small and not clinically relevant.

With regards to endocrine disorders, there have been reports of transitory secondary hypothyroidism probably caused by a decrease in the serum thyroxin-binding globulin due to asparaginase-induced protein synthesis inhibition (see SmPC section 4.8).

Nausea/vomiting are very commonly observed in patients treated with asparaginase-containing treatment regimens but are usually mild. Anorexia, loss of appetite, abdominal cramps, diarrhoea and weight loss have also been reported (see SmPC section 4.8).

Hyperammonaemia has been reported uncommonly in patients treated with asparaginase-containing therapy protocols, especially if patients suffer additionally from hepatic impairment. In very rare cases, severe hyperammonaemia has been reported which may induce neurologic disorders such as seizures and coma (see SmPC section 4.8). Plasma ammonia levels should be determined in all patients with unexplained neurologic symptoms or severe and prolonged vomiting. In case of hyperammonaemia with severe clinical symptoms, therapeutic and pharmacological measures that rapidly reduce plasma ammonia levels (e.g. protein restriction and haemodialysis), reverse catabolic states and increase removal of nitrogen wastes should be initiated and expert advice sought (see SmPC section 4.4). Hyperammonia is included as an important identified risk in the RMP.

Adverse central nervous system reactions observed in patients treated with asparaginase-containing therapy protocols include changes in EEG, seizures, dizziness, somnolence, coma and headache. The causes of these nervous system disorders are unclear. Hyperammonaemia and sinus vein thrombosis may need to be excluded. In rare cases, a reversible posterior leukoencephalopathy syndrome (RPLS) has been observed during therapy with asparaginase-containing regimens (see section 4.8). This syndrome is characterised in magnetic resonance imaging (MRI) by reversible (from a few days to months) lesions/oedema, primarily in the posterior region of the brain. Symptoms of RPLS essentially include elevated blood pressure, seizures, headaches, changes in mental state and acute visual impairment (primarily cortical blindness or homonymous hemianopsia). It is unclear whether the RPLS is caused by asparaginase, concomitant treatment or the underlying diseases. RPLS is treated symptomatically, including measures to treat any seizures. Discontinuation or dose reduction of concomitantly administered immunosuppressive medicinal products may be necessary. Expert advice should be sought (see SmPC section 4.4).

Asparaginase-induced tumour cell destruction may release large amounts of uric acid, resulting in hyperuricaemia. Co-administration of other antineoplastic medicinal products contributes to this effect. Aggressive alkalinisation of the urine and use of allopurinol can prevent urate nephropathy (see SmPC section 4.4). In view of this and although no case of tumour lysis have been reported in the clinical trials, this safety concern has been included in the RMP as a potential risk.

Adverse events with fatal outcome, mainly due to infections, were reported in 4 patients treated with recombinant asparaginase in study MC-ASP.5/ALL (sudden death; disseminated aspergillus opportunistic infection; cerebral aspergillus infection) and in study MC-ASP.2/RHN (septicaemia), whereas no deaths occurred in the asparaginase medac group during the induction phase. Infections (including opportunistic and fungal infections) are identified risks in the RMP that will be closely monitored (see RMP). Concomitant vaccination with live vaccines increases the risk of serious infection. Immunisation with live vaccines should therefore take place at the earliest 3 months after completion of the course of antileukaemic treatment (see SmPC section 4.5). Potential interactions with live vaccines are also adequately addressed in the RMP.

During treatment with asparaginase-containing regimens, myelosuppression, potentially affecting all three cell lines (and infections as mentioned above) can occur. Concomitant treatment with myelosuppressive medicinal products and those known to cause infections are major contributing factors and patients should be carefully monitored for signs and symptoms of myelosuppression and infection (see SmPC sections 4.5 and 4.8).

Regarding the above mentioned sudden death, the Applicant considered metabolic disorders as the likely cause of death even though the autopsy report revealed the presence of liver steatosis. As a toxic reaction

to treatment cannot be excluded, the Applicant correctly considered the event possibly related to recombinant asparaginase.

Most of the patients across clinical studies had alteration in laboratory values. Most were expected and could be attributed to the underlying medical condition or concurrently administered treatments, some known to be associated with significant toxicity. The high incidence of patients administered recombinant asparaginase experiencing an increase of at least 1 CTCAE grade of hyperglycaemia during the induction phase (31.2% vs. 14.9%) was particularly conspicuous. Although hyperglycaemia events are known to occur in patients administered asparaginases, the reasons for the sizeable discrepancy between study arms remains unclear. It is however noted that the majority of the events were grade I events and that a large proportion of both groups at baseline met the criteria for grade I hyperglycaemia. Given these factors and that the events were expected, inclusion of this ADR in the SmPC is considered to be sufficient as regards risk mitigation.

Considering the safety profile of Spectrila, before initiating therapy bilirubin, hepatic transaminases and coagulation parameters (e.g. partial thromboplastin time [PTT], prothrombin time [PT], antithrombin III and fibrinogen) should be determined. After administration of any asparaginase preparation, close monitoring of bilirubin, hepatic transaminases, blood/urinary glucose, coagulation parameters (e.g. PTT, PT, antithrombin III, fibrinogen and D-dimer), amylase, lipase, triglycerides and cholesterol is recommended (see SmPC section 4.4).

Limited data are available in adult patients, particularly those older than 65 years (see RMP). Qualitatively, the same asparaginase-induced adverse drug reactions are observed in adults and children; however, some of these undesirable effects (e.g. thromboembolic events) are known to occur with a higher frequency in adult patients compared to the paediatric population. Because of a higher frequency of comorbidities such as liver and/or renal impairment, patients > 55 years of age usually tolerate asparaginase treatment worse than paediatric patients (see SmPC section 4.8).

Considering the limited data in adults, a single arm PK, PD, safety and immunogenicity study in adult de novo ALL patients will be conducted by the applicant (see RMP). The protocol will be submitted for CHMP review no later than 3 months after the Marketing Authorisation is granted. Analyses of spontaneous reports from adults will also occur and be reported in PSURs.

Women of childbearing potential have to use effective contraception and avoid becoming pregnant while being treated with asparaginase-containing chemotherapy. Since an indirect interaction between components of the oral contraception and asparaginase cannot be ruled out, oral contraceptives are not considered sufficiently safe in such clinical situation. A method other than oral contraceptives should be used in women of childbearing potential. Effective contraception must be used during treatment and for at least 3 months after asparaginase discontinuation (see SmPC section 4.4).

Men should use effective contraceptive measures and be advised to not father a child while receiving asparaginase. The time period following treatment with asparaginase when it is safe to become pregnant or father a child is unknown. As a precautionary measure it is recommended to wait for three months after completion of treatment. However, treatment with other chemotherapeutic agents should also be taken into consideration (see SmPC section 4.6).

There are no data on the use of asparaginase in pregnant women. No reproduction studies in animals with asparaginase were performed but studies with asparaginase preparations in mice, rats, chicken and rabbits have shown embryotoxic and teratogenic effects (see section 5.3). Based on results from animal studies and its mechanism of action, Spectrila should not be used during pregnancy unless the clinical condition of the woman requires treatment with asparaginase (see section 4.6).

It is unknown whether asparaginase is excreted into human breast milk. Because potential serious adverse reactions may occur in nursing infants, Spectrila should be discontinued during breast-feeding (see section 4.6).

No human data on the effect of asparaginase on fertility are available (see SmPC section 4.6).

Safety of Spectrila have not been established in Philadelphia chromosome-positive patients (see SmPC section 4.4).

Spectrila has moderate influence on the ability to drive and use machines, especially through its potential effects on the nervous and gastrointestinal systems (see section 4.8).

No case of asparaginase overdose with clinical symptoms has been reported. There is no specific antidote. Treatment is symptomatic and supportive (see SmPC section 4.9).

Since no data are available on the intramuscular route and extrapolation from intravenous use to the intramuscular route, especially as regards immunogenicity, has not been adequately justified, no recommendation can be made on the intramuscular administration at this stage. Off-label use via intramuscular administration has been included in the RMP as potential safety concern.

Overall, the guidance provided in the SmPC guideline has been adequately followed with regard to addressing the issue of causality and ADRs and relevant ADRs have been included in the SmPC section 4.8.

### 2.6.2. Conclusions on the clinical safety

The safety data provided by the applicant is considered sufficient to characterise the safety profile of Spectrila. The profile of adverse effects of recombinant asparaginase was broadly similar to that of asparaginase medac. The most common side effects are hypersensitivity reactions, hyperglycaemia, hypoalbuminaemia, nausea, vomiting, diarrhoea, abdominal pain, oedema, fatigue, and change in laboratory parameters (e.g. transaminases, bilirubin, blood lipids and coagulation parameters).

To further characterise the safety of Spectrila in adults a single arm PK, PD, safety and immunogenicity study in adult de novo ALL patients will be conducted by the applicant (see RMP).

## *2.7. Risk Management Plan*

The CHMP received the following PRAC Advice on the submitted Risk Management Plan (RMP).

The PRAC considered that the RMP version 1.0 (dated 26 August 2013) could be acceptable if the applicants implements all the changes to the RMP as detailed in the PRAC endorsed PRAC Rapporteur assessment report.

The CHMP endorsed this advice.

The applicant implemented the changes in the RMP as requested by PRAC and CHMP.

The CHMP endorsed the RMP version 10.0 (dated 24 November 2015) with the following content:

***Safety concerns***

**Table 62: Summary of the Safety Concerns**

| Important identified risks | – Infections (including opportunistic and fungal infections)<br>– Severe hypersensitivity reactions<br>– Decreased asparaginase activity<br>– Coagulation factor deficiencies<br>– Haemorrhage<br>– Thromboembolic events<br>– Hepatotoxicity<br>– Pancreatitis<br>– Neurotoxicity<br>– Hyperglycaemia<br>– Changes in blood lipids<br>– Hyperammonaemia<br>– Embryotoxic and teratogenic effects<br>– Schedule-dependent interaction with methotrexate<br>– Schedule-dependent interaction with Ara-C<br>– Interaction with glucocorticoids (altered coagulation and increased risk of osteonecrosis) |
|---|---|
| **Important potential risks** | – Reversible posterior leukoencephalopathy syndrome (RPLS)<br>– Tumour lysis syndrome<br>– Off-label use via IM route<br>– Potential interaction of vincristine and ASNase<br>– Potential interaction with anticoagulants<br>– Potential interaction with other medicines where impaired liver metabolism could increase toxicity<br>– Potential interaction with live vaccines |
| **Missing information** | – Safety in patients > 65 years of age |

***Pharmacovigilance plan***

Table of ongoing and planned additional pharmacovigilance studies/activities in the Pharmacovigilance Plan

| Study (type and study number) | Objectives | Safety concerns addressed | Status (planned, started) | Date for submission of interim or final reports |
|---|---|---|---|---|
| Clinical phase IV study MC-Spectrila.1/ALL | Assessment of PK, PD, safety and immunogenicity of Spectrila after repeated-dose treatment of patients with de novo ALL. | Safety of Spectrila in adult patients | Planned | Interim reports will be provided in the RMP updates and regular PSURs, together with analyses of spontaneous reports from adults treated with |

| Study (type and study number) | Objectives | Safety concerns addressed | Status (planned, started) | Date for submission of interim or final reports |
|---|---|---|---|---|
| | | | | Spectrila in other study protocols.<br>Final report will be submitted approximately 35 months after granting MA |

The PRAC also considered that routine pharmacovigilance is sufficient to monitor the effectiveness of the risk minimisation measures.

***Risk minimisation measures***

**Table 63: Summary table of Risk Minimisation Measures**

| Safety concern | Routine risk minimisation measures | Additional risk minimisation measures |
|---|---|---|
| Infections (including opportunistic and fungal infections) | *SmPC text in section 4.5, 4.8*<br>*Perscription only medicine* | None |
| Severe hypersensitivity reactions | *SmPC text in section 4.3, 4.4, 4.8, 5.1,* 6.1.<br>*Perscription only medicine* | None |
| Decreased asparaginase activity | *SmPC text in section 4.2, 4.3, 4.4.*<br>*Perscription only medicine* | None |
| Coagulation factor deficiencies | *SmPC text in section 4.3, 4.4, 4.5, 4.8.*<br>*Perscription only medicine* | None |
| Haemorrhage | *SmPC text in section 4.3, 4.4, 4.5, 4.8.*<br>*Perscription only medicine* | None |
| Thromboembolic events | *SmPC text in section 4.3, 4.4, 4.5, 4.8.*<br>*Perscription only medicine* | None |

| **Safety concern** | **Routine risk minimisation measures** | **Additional risk minimisation measures** |
|---|---|---|
| Hepatotoxicity | *SmPC text in section 4.2, 4.3, 4.4.* 4.5, 4.8, *5.1.*<br>*Perscription only medicine* | None |
| Pancreatitis | *SmPC text in section 4.3, 4.4, 4.8.*<br>*Perscription only medicine* | None |
| Neurotoxicity | *SmPC text in section 4.8, 5.1.*<br>*Perscription only medicine* | None |
| Hyperglycaemia | *SmPC text in section 4.4, 4.8.*<br>*Perscription only medicine* | None |
| Changes in blood lipids | *SmPC text in section 4.4, 4.8.*<br>*Perscription only medicine* | None |
| Hyperammonaemia | *SmPC text in section 4.4, 4.8.*<br>*Perscription only medicine* | None |
| Embryotoxic and teratogenic effects | *SmPC text in section 4.6, 5.3.*<br>*Perscription only medicine* | None |
| Schedule-dependent interaction with methotrexate | *SmPC text in section 4.5.*<br>*Perscription only medicine* | None |
| Schedule-dependent interaction with Ara-C | *SmPC text in section 4.5.*<br>*Perscription only medicine* | None |
| Interaction with glucocorticoids (altered coagulation and increased risk of osteonecrosis) | *SmPC text in section 4.4, 4.5.*<br>*Perscription only medicine* | None |
| **Important potential risks** | | |
| Reversible posterior leukoencephalopathy syndrome (RPLS) | *SmPC text in section 4.4, 4.8.*<br>*Perscription only medicine* | None |
| Tumour lysis syndrome (TLS) | *SmPC text in section 4.4.*<br>*Perscription only medicine* | None |
| Off-label use via IM route | *SmPC text in section 4.2.*<br>*Perscription only medicine* | None |

| Safety concern | Routine risk minimisation measures | Additional risk minimisation measures |
|---|---|---|
| Potential interaction of vincristine and ASNase | *SmPC text in section 4.5.*<br>*Perscription only medicine* | None |
| Potential interaction with anticoagulants | *SmPC text in section 4.5.*<br>*Perscription only medicine* | None |
| Potential interaction with other medicines where impaired liver metabolism could increase toxicity | *SmPC text in section 4.4, 4.5, 4.8*<br>*Perscription only medicine* | None |
| Potential interaction with live vaccines | *SmPC text in section 4.5.*<br>*Perscription only medicine* | None |
| **Missing information** | | |
| Safety in patients > 65 years of age | *SmPC text in section 4.2.*<br>*Perscription only medicine* | None |

## *2.8. Pharmacovigilance*

**Pharmacovigilance system**

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

## *2.9. Significance of paediatric studies*

The CHMP is of the opinion that studies MC-ASP.4/ALL, MC-ASP.5/ALL, and MC-ASP.6/INF, which are contained in the agreed Paediatric Investigation Plan, P/0084/2013 and were completed after 26 January 2007, are considered as significant.

The assessment criteria for significance of studies as defined in Section III, Title 4.2 of the Europe Commission Communication – "Guideline on the format and content of applications for agreement or modification of a paediatric investigation plan and requests for waivers or deferrals and concerning the operation of the compliance check and on criteria for assessing significant studies (2014/C 338/01)" has been fulfilled, taking into account the following:

Studies MC-ASP.5/ALL and MC-ASP.4/ALL are both comparative efficacy studies: Study MC-ASP.5/ALL was a multicentre, randomised, active-controlled, double-blind, parallel-group phase III study designed to assess the efficacy and safety of recombinant L-asparaginase in comparison to Asparaginase medac during treatment of children with de novo ALL; Study MC-ASP.4/ALL was a single-centre, randomised, double-blind, parallel group phase II clinical trial to compare the pharmacokinetics, pharmacodynamics, efficacy, and safety of recombinant ASNase versus Asparaginase medac during induction treatment in children with de novo ALL.

In addition, study MC-ASP.6/INF, a non-controlled multicentre phase II study of recombinant asparaginase for first-line treatment in infants (< 1 year of age at diagnosis) with de novo ALL, provided meaningful pharmacokinetic/pharmacodynamic data.

## *2.10. Product information*

### 2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

# 3. Benefit-Risk Balance

***Benefits***

**Beneficial effects**

The pharmacodynamic and pharmacokinetic parameters were the main outcome measures in support of the clinical efficacy. Recombinant asparaginase (Spectrila) was compared to asparaginase medac in two pivotal trials, a phase III study (MC-ASP.5/ALL) and a phase II pharmacokinetics study (MC-ASP.4/ALL) in children with previously untreated acute lymphoblastic leukaemia. Supportive data were available from two additional pharmacokinetic studies in patients with relapsed haematological neoplasias (MC-ASP.2/RHN), in adult patients with relapsed acute lymphoblastic leukaemia (MC-ASP.1/ALL) and one efficacy study in infants with previously untreated acute lymphoblastic leukaemia (MC-ASP.6/INF).

Data from all clinical studies with intravenous recombinant asparaginase showed swift, sustained and complete reductions in serum and CSF asparagine. In the main study MC-ASP.5/ALL, complete asparaginase depletion in serum was 94.9% in the rASNase arm versus 94.1% in the asparaginase medac arm and the confidence intervals for the risk difference fell entirely within the predefined interval of [-10%, 10%]: 0.8% [-6.25%; 8.04%], p-value= 0.0028.

The pivotal study data on asparagine depletion were supported by slight decreases in serum glutamine levels and increases in serum aspartic acid and by observed mean serum trough asparaginase activities >100 U/L, which is used in clinical practice as the threshold level for assuring complete asparaginase depletion in serum and CSF in patients.

Data from study MC-ASP.5/ALL were also presented where the observed changes in the serum amino acid concentrations were similar in both asparaginase study arms. Comparable trends were additionally observed in study MC-ASP.4/ALL, with the exception of glutamine levels both in CSF and serum.

With regards to efficacy endpoints, most patients achieved complete remission (91.8% in the rASNase arm versus 96% in ASNas medac arm) and relapse- and event-free survival rates were high (96.9% at 12 months and 96.9% at 24 months for rASNase versus 100% and 96.4% for ASNase medac respectively) as expected for current treatment protocols for paediatric ALL in which asparaginase is administered. Overall, rates were similar between the study arms of the pivotal and PK studies. In the small study in infants all patients had complete remission after induction treatment and almost all had achieved a positive MRD status. For adults, the survival and relapse data could not be interpreted as patient numbers were too low.

### Uncertainty in the knowledge about the beneficial effects

Within the pivotal and supportive studies, whilst the data for the primary and secondary PD outcomes appeared broadly similar, there were signals in the PK and PD data which suggested that as treatment continued there was a trend to better outcome, even though small, with asparaginase medac treatment compared to treatment with recombinant asparaginase. A potential source of the differences is the large difference in aggregate component between the comparator products, (asparaginase medac 20.5% vs. recombinant asparaginase 0.36%). Available data suggest that the aggregates are associated with higher enzymatic activity than the tetramers within the product; hence, the activity of asparaginase medac could be expected to be higher. Additional PK and PD data submitted could not be considered robust and trends to slightly better efficacy seen in patients administered asparaginase medac were considered by the CHMP to be of relatively low importance.

Limited data were presented in certain populations and settings with the use of Spectrila. These include use in adults, infants and relapsed disease and use of other asparaginases after allergic/ hypersensitivity reaction to recombinant asparaginase. However, considering available post marketing information and the clinical experience with the use of asparaginases in treatment protocols for these groups, it is considered available safety and efficacy data may be extrapolated to adults, infants and post-induction settings. In addition, the Applicant will undertake further evaluation of PK, PD and safety (including immunogenicity) in adults (see RMP).

***Risks***

### Unfavourable effects

The profile of adverse effects of recombinant asparaginase was broadly similar to that of asparaginase medac. Events of thrombosis, pancreatitis, infection and liver function changes were assessed and no notable differences were indicated. The main adverse events of interest are the formation of anti-drug antibodies and allergic/hypersensitivity reactions. Approximately 50% of patients had developed antibodies by the end of the asparaginase induction treatment in the pivotal study, with approximately half developing concomitant decrease in asparaginase activity, suggesting the presence of neutralising ADAs. There were no clear differences seen between study arms for these safety endpoints. This was also the case for the post induction phase, although data were limited.

Data on allergic reactions, which included all allergic reactions occurring within the 24 hours after an infusion were provided and suggested a slightly lower proportion of patients suffering allergic reactions in the recombinant asparaginase arm (18% vs 25%) during the induction phase. Overall, the incidence of allergic reactions in both study arms were significantly increased when compared to the protocol mandated analysis which restricted evaluation to any allergic reaction event occurring within 12 hours of study drug administration.

Hypersensitivity reactions were similar between both arms with an incidence of approximately 36-39%. The incidence of hypersensitivity reactions together with the efficacy of rASNase were also assessed in 12 infants patients (≤ 1 year) in the uncontrolled phase II study, MC-ASP.6/INF. Silent inactivation was reported in 4 out of the 12 enrolled patients (33%), even though no ADAs were detected in any patients. Asparaginase activity levels lower than that postulated to assure complete asparagine depletion (100 U/L) were detected at some time points in all patients but one, with a higher than expected variability between patients. Nevertheless, complete asparaginase depletion was obtained in all but one patient.

### Uncertainty in the knowledge about the unfavourable effects

The anti-drug antibody assay was reasonably well performed, although it has some limitations. In particular, it cannot be excluded that the ADA assay underestimates the incidence and titre of ADAs due to interference with ADA determination by asparaginase. Decreased asparaginase activity has been

included as an important identified risk in the RMP. Furthermore, treatment may be monitored based on the trough serum asparaginase activity measured three days after administration of Spectrila. If asparaginase activity values fail to reach target levels, a switch to a different asparaginase preparation could be considered (see SmPC section 4.4).

There are few safety data available in adults from the development programme. Generally, response and survival outcomes in adults do not approximate those in children and tolerability of paediatric doses of asparaginase is poor due to adverse effects. The SmPC reflects how the safety profiles in adults and children differ (see SmPC section 4.8). Further evaluation of PK, PD and safety in adults will be conducted post-authorisation (see RMP).

***Benefit-risk balance***

**Importance of favourable and unfavourable effects**

Complete asparagine depletion is considered as a valid surrogate for clinical efficacy of asparaginases and was considered an adequate endpoint to compare Spectrila with ASNase medac. Asparagine levels decreased after the first dose of either ASNase preparation and remained depleted until end of ASNase treatment. Non-inferiority of Spectrila to asparaginase medac has been shown on the basis of asparaginase depletion.

Although lower ASNase activity was observed for Spectrila versus ASNase medac, ASNase trough serum activity levels as low as 10-20 U/L led to complete ASN depletion in serum and CSF based on the provided data and supporting literature data showed that lower levels than 100 IU/L are sufficient (Boos et al. 1996 (BFM group); Rizzari et al. 2000 (AIEOP group); Wetzler et al. 2007 (CALGB)).

The profile of adverse effects of recombinant asparaginase was broadly similar to that of asparaginase medac and all important identified and potential risks have been adequately addressed in the Risk Management Plan.

**Benefit-risk balance**

The benefit-risk balance of Spectrila as a component of antineoplastic combination therapy for the treatment of acute lymphoblastic leukaemia (ALL) in paediatric patients from birth to 18 years and adults is considered positive.

***Discussion on the benefit-risk balance***

Non-inferiority of Spectrila to asparaginase medac was shown on the basis of asparaginase depletion.

Very few clinical efficacy data are available in adult ALL patients treated with recombinant asparaginase, as the adult studies were terminated early. However, given that the experience with the use of asparaginases in the adult ALL population and that any PK and PD differences seen between test and reference products in children are not expected to differ significantly from those that might occur in adults given similar doses, the use of Spectrila can be extended to adults. PK, PD, safety and immunogenicity data will be collected post-approval in a single-arm adult de novo ALL patients study (see RMP).

# 4. Recommendations

***Similarity with authorised orphan medicinal products***

The CHMP by consensus is of the opinion that Spectrila is not similar to Atriance (nelarabine), Evoltra

(clofarabine), Iclusig (ponatinib), Sprycel (dasatinib), Xaluprine (mercaptopurine), Blincyto (blinatumomab) within the meaning of Article 3(3)(b) of Commission Regulation (EC) No. 847/2000. See appendix 1.

***Outcome***

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Spectrila as a component of antineoplastic combination therapy for the treatment of acute lymphoblastic leukaemia (ALL) in paediatric patients from birth to 18 years and adults is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

***Conditions or restrictions regarding supply and use***

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

***Conditions and requirements of the Marketing Authorisation***

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

***Conditions or restrictions with regard to the safe and effective use of the medicinal product***

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

***Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States***

Not applicable.

***Paediatric Data***

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0084/2013 and the results of these studies are reflected in the Summary of Product

Characteristics (SmPC) and, as appropriate, in the Package Leaflet.

In accordance with Article 45(3) of Regulation (EC) No 1901/2006, significant studies in the agreed paediatric investigation plan P/0084/2013 have been completed after the entry into force of that Regulation.

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