15 September 2016
EMA/CHMP/238678/2017
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Graspa

International non-proprietary name: eryaspase

Procedure No. EMEA/H/C/004055/0000

Note

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

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List of abbreviations

ALL	Acute Lymphoblastic Leukemia
ANC	Absolute Neutrophil Count
AUC	Area Under the Curve
BM	Bone Marrow
CHMP	Committee for Medicinal Products for Human Use
CNS	Central Nervous System
CR	Complete Remission
CSF	Cerebrospinal Fluid
DFS	Disease Free Survival
DSMB	Data Safety Monitoring Board
EAP	Expanded Access Program
EFS	Event-free Survival
EMA	European Medicines Agency
Gln	Glutamine
Glu	Glutamic acid
IBFM	International Berlin Munich
i.m.	intramuscular
i.v.	intravenous
MAA	Marketing Authorization Application
OS	Overall Survival
PD	Pharmacodynamic
PDCO	Pediatric Committee
Ph-	Philadelphia Chromosome Negative
PIP	Pediatric Investigation Plan
PK	Pharmacokinetics
RBC	Red Blood Cell
RFS	Relapse Free Survival
WHO	World Health Organization

1. Recommendation

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for Graspa, an orphan medicinal product in the treatment of paediatric and adult patients with acute lymphoblastic leukaemia that has either relapsed or failed first line treatment; Also for the treatment of paediatric and adult patients with acute lymphoblastic leukaemia and hypersensitivity to asparaginase, **is not approvable** since "major objections" still remain, which preclude a recommendation for marketing authorisation at the present time.

The major objections precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

Quality

Drug substance

New source of L-asparaginase - comparability between native and recombinant L-asparaginase should be demonstrated. In addition, the applicant should confirm that only L-asparaginase Spectrila is going to be used in the manufacture of Eryaspase, and revise the dossier accordingly (references to E. coli L-asparaginase have been found, which create confusion on which type the Applicant is referring to).

Drug product

Stability – a shelf life of 120hr at 2-8°C shelf-life may be supportable however further justification is required. The shelf-life appears somewhat arbitrary and at present does not appear to be set based on levels of impurities administered to patients (particularly extracellular asparaginase).

GMP

The current manufacturing authorisation/GMP certificate uploaded in EudraGMDP and mentioned in the dossier is only for IMPs. Accordingly, the applicant needs to provide an updated manufacturing authorisation covering human medicinal products.

Clinical

1. The validity of the comparison based on PD parameters, either asparagine depletion or asparaginase activity, is seriously questioned and thus the non-inferiority in the activity of Graspa vs native L-asparaginase cannot be established. In the absence of reliable PD parameters, further discussion is needed on the robustness of the conventional clinical endpoints from the pivotal study in order to demonstrate the therapeutic efficacy of Graspa.

2. A new source of L-asparaginase is proposed for use in the updated dossier. In addition to the comparability analysis conducted at the quality level, the Applicant is requested to discuss on the relevance of the clinical studies submitted (using native L-asparaginase encapsulated in RBCs) to the new formulation (containing recombinant L-asparaginase), including the need to conduct additional non-clinical and/or clinical bridging studies,

3. The current wording of the indication allows using Graspa with any chemotherapeutic regimen, though only data for COOPRALL protocol are available. The Applicant should discuss how the results of the pivotal study can be reasonably generalised to these other protocols.

New active substance

The biological substance contained in Graspa, either native L-asp or recombinant L-asparaginase, is not considered to qualify as a NAS.

Proposal for questions to be posed to additional experts

Proposal for inspection

GMP inspections

No GMP issues Identified.

GCP inspections

A request for GCP inspection has been adopted for the following clinical study; GraspaLL 2009-06.

This was a triggered GCP inspection. The criteria for selection of this application included the indication, the fact that the trial was performed on pediatric population, and that no inspection had been performed by EU inspectorates on this application. In addition, issues had been identified at the central laboratory used for this trial during a previous inspection and a re-inspection of the laboratory was then recommended by the inspection team.

There were some deficiencies with the informed consent at the two investigator sites inspected. These were classified as major and although may have affected patient's rights, they do not affect the quality and acceptability of the data.

Revised data of asparaginase activity measurements are available and were requested for submission by the applicant in their response to the D120 list of questions for assessment. It was noted at the time that the revised asparaginase activity data may therefore be accepted when submitted, if the assessors consider that the limits of ± 15 % for precision and accuracy, commonly used for the measurement of concentrations of analytes of chemical origin, are not required in this case and that wider acceptance limits are acceptable. The Rapporteurs, following assessment, consider these limits acceptable although further clarification is requested on the actual valid data.

New active substance status

From a Quality perspective, eryaspase is not considered to be a new active substance. The active substance in this product is considered to be the L-asparaginase, which cannot be regarded as a new active substance since it is bought from a commercial supplier ready for incorporation into the RBCs and the commercial source already has a Marketing Authorisation licence within the EU. Incorporation into the RBCs is regarded as a formulation procedure and not relevant for the consideration of NAS status. Consequently, it has not been shown that the L-asparaginase in Graspa differs significantly from the already licensed product with regard to safety and/or efficacy due to differences in one or a combination of the following: molecular structure, nature of the source material or manufacturing process. The change from native to recombinant L-asparaginase proposed in the updated dossier does not change this conclusion.

For L-asparaginase in eryaspase to qualify as a NAS the Applicant should substantiate their claim based on non-clinical/clinical data; which ***should be linked*** to a difference in either structure, source material and/or the manufacturing process.

2. Executive summary

2.1. About the product

Graspa is a personalized dispersion for infusion of eryaspase (proposed INN) in a preservative solution (SAG-Mannitol [SAG-M]).

According to the applicant, the active substance is “eryaspase”, a complex substance manufactured from commercially available L-asparaginase, derived from E. coli (a marketing authorization licence to Medac was granted in Germany) and from compatible erythrocytes qualified for transfusion applications. Its common name is L-asparaginase encapsulated in erythrocytes.

The erythrocytes in Graspa ‘pump’ asparagine from blood plasma through sodium-coupled neutral amino acid transporters (SNAT 3/5) into the erythrocyte interior and the enzymatic activity of entrapped L-asparaginase cleaves the entrapped L-asparagine into aspartic acid and ammonium. This leads to plasmatic asparagine depletion and starvation of the leukemic cells. In contrast to normal cells, lymphoblastic tumour cells have a very limited capacity of synthesising asparagine because of a significantly reduced expression of asparagine synthetase. Therefore, these tumour cells require exogenous supply with asparagine that can only be obtained by diffusion from the environment outside the cell. As a result of asparaginase-induced asparagine depletion in serum, protein synthesis in lymphoblastic tumour cells is disturbed while sparing most normal cells.

The objective of the encapsulation of the L-asparaginase is to extend its life and activity. In addition, being encapsulated prevents immune responses against the enzyme.

During the MA procedure, the Applicant has updated the quality dossier and now proposes to use the commercially available recombinant L-asparaginase as the source enzyme, instead to the previously proposed native L-asparaginase, to be encapsulated in erythrocytes.

The proposed clinical indication is:

“Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of pediatric and adult patients with acute lymphoblastic leukaemia that has either relapsed or failed first line treatment. Graspa is also indicated for the treatment of pediatric and adult patients with acute lymphoblastic leukaemia and hypersensitivity to asparaginase.”

As part of the responses to the D120 LOQ, a revised indication is proposed:

“Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of children over 1 year of age and adult patients with Philadelphia chromosome negative acute lymphoblastic leukaemia, who have either relapsed or failed first line treatment”.

2.2. The development programme/compliance with CHMP guidance/scientific advice

The following CHMP scientific advice was provided:

- EMEA/H/SA/991/1/2007/PA/SME/III
- EMEA/H/SA/991/1/FU/1/2010/PA/SME/ADT/1
- EMEA/H/SA/991/2/2012/PA/ADT/SME/I
- EMEA/H/SA/991/1/FU/2/2014/PA/SME/III

In terms of quality there are deviations from the scientific advice provided. Where there were changes in the manufacturing process comparability was requested however this has not been conducted. The Applicant sought advice on their claim of new active substance (NAS). This was not supported by CHMP who considered the product to contain a known active substance. The Applicant would need to show that they had created a new substance; for instance one with a different molecular structure such as a change in amino acid sequence, or covalent bonding to the erythrocyte etc…. The Applicant has submitted a claim for NAS within this MAA nonetheless. It is also noticed that the shelf-life proposed during development is different from the shelf-life now requested, which requires further justification.

The main clinical advice was provided during the 2008 and 2014 procedures.

Number of days with asparagine depletion was initially accepted as the primary endpoint for the comparative pharmacological study. However it was later agreed with the Applicant for asparaginase activity to serve as the primary endpoint, as the Applicant reported issues with the reliability assay related to the ex-vivo depletion of asparagine. The CHMP requested that the method for measuring asparaginase activity be validated. The same primary endpoint was to be used for both study arms.

The CHMP agreed with the Applicant's request on ethical grounds not to undertake CSF asparagine and asparaginase measurements. Also the need to provide clinical outcome data prior to licensing was agreed not to be necessary. With regard to the indications sought, the proposed indication of patients with first relapse was considered acceptable. To support an indication in patients who cannot use conventional asparaginases due to hypersensitivity reactions and/ or high levels of circulating neutralising antibodies, the applicant was asked to perform a single arm trial enrolling such patients.

2.3. General comments on compliance with GMP, GLP, GCP

GMP

Compliance with GMP is required. The Applicant should list all the manufacturing and control sites involved in the complete manufacturing process of eryaspase (including now the L-asparaginase as drug substance) and relevant documentation, fully updated GMP certificate(s) for commercial manufacture should be provided in Module 1 and Module 3 as appropriate.

GLP

The definitive safety studies were conducted in compliance with Good Laboratory Practice.

GCP

All clinical studies are claimed by the Applicant to be conducted in accordance with ICH GCP. A request for GCP inspection has been adopted for the following clinical study; GraspaLL 2009-06. (see summary of the IIR)

2.4. Type of application and other comments on the submitted dossier

Legal basis

This is a full application through the centralised procedure, according to Regulation (EC) No 726/2004 Article 3(1) Annex 4 (Orphan designated medicinal product) in accordance with Article 8(3) in directive 2001/83/EC for a new active substance.

- **Conditional approval / Approval under exceptional circumstances**

N/A

- **Accelerated procedure**

This was rejected on grounds that the conditions for accelerated assessment were not considered to have been adequately met. The Applicant did not address how Graspa could be shown to be of major therapeutic benefit over existing asparaginase treatments (not only L-asparaginase) in previously untreated, relapsed or recalcitrant ALL or address the benefits of Graspa in relapsed or recalcitrant disease when treatment with currently licensed asparaginases was not an option.

- **Biosimilar application**

N/A

- **1 year data exclusivity**

N/A

- **Significance of paediatric studies**

The applicant ERYTECH Pharma S.A. submitted a request to the PDCO on 30 March 2015 to check as to whether the studies conducted are in compliance with the agreed paediatric investigation plan of 24 November 2014. The PDCO however could not confirm that the applicant's study GraspaLL2009-06 had been completed in compliance with all key elements for study 4 in the Opinion. Specifically, the PDCO is of the view that for the primary endpoint, no assessments have been made that are in accordance with the requirements as per the Opinion. Furthermore, an additional interim data analyses was conducted. Both these issues are understood to be recognised by the applicant. These matters require further scientific discussion and a regulatory implementation.

The PDCO was informed about a teleconference of the EMA on 4 June 2015 during which procedural options in this ongoing procedure as well as options and consequences for the initial marketing authorisation application (originally planned for 6 July 2015) were briefly discussed. A pre-submission meeting of the applicant with the forthcoming CHMP Rapporteurs had taken place on 7 April 2015 and the applicant provided their minutes to EMA and the PDCO Rapporteur.

The PDCO discussed an interaction with the CHMP Rapporteurs on the scientific and regulatory aspects.

The PDCO finalised on 19 June 2015 this partially completed compliance procedure and confirmed the compliance of only study(ies) 1 and 3 in the agreed paediatric investigation plan.

3. Scientific overview and discussion

3.1. Quality aspects

According to the Applicant, the active substance of Graspa is “eryaspase”, a complex substance manufactured from a biological starting material commercially available L-asparaginase produced in *Escherichia coli* and from compatible erythrocytes qualified for transfusion applications by a blood establishment. Its common name is L-asparaginase encapsulated in erythrocytes. The CHMP does not agree with this definition of the active substance, and considers L-asparaginase as the drug substance in Graspa. However, this report will follow the current structure of the MAA submitted by the Applicant. In the current submission, the L-asparaginase has been changed to the recombinant form centrally-approved under the name of Spectrila. The consideration of the enzyme as the active substance has not changed.

The drug product Graspa, as defined by the Applicant, is a personalized dispersion for infusion of eryaspase (proposed INN) in a preservative solution. It is presented in a hemocompatible container for transfusion applications. Graspa is a ready to use product administered by intravenous infusion.

Graspa is described as a personalized medicinal product manufactured according to the physician specific prescription issued for each individual patient following two principal dosing regimens. The allogeneic red blood cell (RBC) starting material is selected to be compatible with the patient immunologic and hematologic profile. The volume of Graspa is adapted so that the total amount of activity prescribed is delivered to the patient (the total volume of the bag of the finished product is injected).

3.1.1. Active Substance

General Information

Eryaspase is constituted of erythrocytes encapsulating L-asparaginase at a range between 78 and 146 IU/ml of eryaspase. The erythrocytes and the L-asparaginase properties remain similar after the encapsulation process. The key elements of this active substance are:

- L-asparaginase moiety which accounts the L-asparaginase activity. L-ASNase is an enzyme obtained from *Escherichia coli (E. coli)*. In the updated dossier, a recombinant enzyme is used.

- The erythrocytes, which protect the enzyme from the extracellular environment (expected to achieve a longer life-span in the blood stream).

Graspa Bulk Drug Substance (BDS) dispersion consists in eryaspase (L-ASNase encapsulated in erythrocytes) a preservative solution.

This product aims to combine the capacity of erythrocytes to 'pump' asparagine from blood plasma through sodium-coupled neutral amino acid transporters 3 and 5 (SNAT3/5) and the enzymatic activity of entrapped L-asparaginase to cleave the intra-erythrocyte L-asparagine into ammonium and aspartic acid which can be released to the blood stream by passive diffusion.

The Applicant was asked to provide evidence that asparagine does in fact enter the RBC, which has been provided, but questions remain with regard to the proposed mechanism of action. The purpose of the encapsulation is to increase the plasma half-life/activity of L-asparaginase and also reduce unwanted immune reactions to the enzyme, both limitations of current treatment (standard enzyme-replacement therapy with 'unencapsulated' L-asparaginase). Enzyme specific activity is maintained within the RBC.

Manufacture, characterisation and process controls

Description of manufacturing process and process controls

The process is designed as a continuous process from the DS starting material to the DP. Hence, the DS is produced as a Bulk DS (BDS) processed directly into DP with no isolated stage. A general flowchart has been provided that includes information on process parameters/operational ranges and control methods. For each prescription received, a specific batch is manufactured independently for the specific patient needs from a single bag of pRBC.

Each patient specific batch is prepared independently according to a process that is broken down into five main stages:

- Washing of erythrocytes

- Introduction of L-asparaginase

- Encapsulation

- Incubation

- Washing

Finally, the eryaspase Drug Substance is directly formulated with the excipient solution to prepare Graspa's Bulk Drug Substance (BDS).

Control of materials

All the materials used by ERYTECH Pharma are obtained from qualified suppliers. In accordance with the conclusions of each supplier qualification program and a risk based approach method, ERYTECH Pharma rely on the supplier CoA and identity testing of the materials to ensure each material meets the specifications described in this section.

Control of critical steps and intermediates

Tests and acceptance criteria performed at critical steps of the aseptic manufacturing process of eryaspase have been described.

Process validation

The validation of Graspa manufacturing process is supported by several elements. All IPCs and all Drug Substance specifications are monitored in order to confirm that all the product Critical Quality Parameters are within the approved ranges.

Characterisation

The elucidation of the structure of eryaspase consists mainly in the verification that the characteristics of the erythrocytes and of the L-asparaginase that constitute eryaspase keep their properties after the manufacturing process.

ERYTECH Pharma verifies that the method of encapsulation of L-asparaginase in erythrocytes used by ERYTECH does not alter the following erythrocyte properties:

- The hematological characteristics
- The oxygen function of the erythrocytes
- The erythrocyte surface antigens
- Erythrocyte's formability (Ektacytometry)
- The cell quality (size and granularity) compared to source cells.

Stability studies have also been used to characterize the functionality of the RBC, with examination of extracellular haemoglobin, ATP and 2-3 DPG (metabolic activity), encapsulated L-asparaginase and haematological parameters.

The Applicant indicates that previous studies have shown that encapsulation did not affect the functional properties of L-asparaginase in the RBCs.

The limits for the main potential impurities have been established taken into consideration the level that was found safe in the literature.

Specification

Due to the continuous nature of the manufacturing process identical testing is performed for release of eryaspase and DP so the release testing is further discussed under control of drug product below.

The Analytical Procedures used are described and validated in the Drug Product section.

Batch analyses results of the Drug Substance and Drug Product manufacturing process validation lots are provided in the Drug Product section.

There are no established reference standards for enzymes encapsulated in erythrocytes to date. In order to provide a suitable reference for eryaspase activity test, which was specifically developed for the need of Graspa, an in-house reference standard had to be developed by ERYTECH Pharma using the Bulk Drug Substance.

Stability

As the manufacture from the claimed drug substance (eryaspase) to drug product is continuous the Applicant has not performed stability studies with the Drug Substance. Stability studies were carried out with the Drug Product.

3.1.2. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Graspa Drug Product is a dispersion for infusion of eryaspase formulated in a preservative solution. It is presented in a haemocompatible bag suitable for the preparation of infusions of blood products.

The starting material of Erythrocytes is obtained via allogeneic blood donation.

Graspa was developed taking into account the current practices and standards followed by blood establishments for the administration of erythrocyte preparations. The preservative solution and haemocompatible bags used for the manufacture of the Drug Product are standalone medical devices commercialized under their CE marking for use by blood establishments and hospital transfusion units for the preparation of leukoreduced RBCs.

Graspa is a personalized medicinal product manufactured according to the physician specific prescription issued for each individual patient. In accordance with the therapeutic practices for the treatment of ALL and its diagnosis, physicians prescribe a determined amount of L-asparaginase activity following two principal dosing regimens.

No changes into Graspa formulation have occurred during its development.

Manufacture of the product and process controls

ERYTECH Pharma's facilities were established to support the development of Graspa.

Excipients

The excipient is a preservative solution which has a CE marking which supports its use for blood storage solution application. ERYTECH Pharma relies on tests performed by its supplier. An additional control is performed at reception for the identification of the product based on the verification of the presence of glucose by a physico-chemical method.

Product specification

The DP specifications include tests for identity and content. Haematological tests are included.

Impurities have been described. All primary packaging materials comply with the Ph. Eur. standards. Shipment has been suitably qualified. Each supplier is validated in an appropriate manner.

Stability of the product

Stability of Graspa was mainly studied in real time/real temperature studies in order to simulate the actual in-use storage conditions.

Adventitious agents

Control with respect to adventitious agents is based on a process risk assessment. The process is performed aseptically with closed sterile procedures and single-use disposables. Raw materials are obtained sterile and pyrogen free from qualified suppliers. The blood donation is obtained from an authorised blood establishment. The Applicant has provided details of the donation and testing process, with standard exclusion criteria (details in dossier). The only materials of human or animal origin are the starting materials pRBC donation and recombinant L-asparaginase. The RBC's supplied are compliant with current regulatory requirements. L-asparaginase use is justified by the Applicant who states it is a licensed medicinal product made in accordance with GMP principles and in *E.coli* which do not harbour mammalian viruses. In addition, the manufacturer has provided a statement to say it is compliant with current TSE guidelines.

GMO

N/A

3.1.3. Discussion on chemical, pharmaceutical and biological aspects

Active Substance

The CHMP disagrees with the definition of drug substance for this medicinal product, in line with previous advice provided by the CHMP and CAT to the Applicant and the Applicant is requested to re-configure the dossier to reflect this. On their response document, the Applicant insists on defining the active substance as the encapsulated enzyme and presents results to support the claim. However, the conclusion remains the same (see discussion on New Active Substance).

Drug Substance Eryaspase, as defined by the Applicant, is constituted of erythrocytes encapsulating L-asparaginase. Graspa Bulk Drug Substance (BDS) dispersion consists in the Drug Substance eryaspase (L-ASNase encapsulated in erythrocytes) in a preservative solution.

This product combines the capacity of erythrocytes to 'pump' asparagine from blood plasma and the enzymatic activity of entrapped L-asparaginase to cleave the intra-erythrocyte L-asparagine into ammonium and aspartic acid which can be released to the blood stream by passive diffusion. The Applicant was asked to provide evidence to substantiate the proposed mechanism of action (i.e. asparagine enters the RBC, rather than slow release of L-asparaginase in vivo as the RBC lyse). Although the evidence presented indicate that the enzyme does indeed enter the RBC *ex vivo*, it remains to be shown whether the *in vivo* effect occurs inside the RBC or through enzyme release to plasma.

Information has been provided on the manufacturer and the manufacturing process and process controls. The process is continuous from Drug Substance to Drug Product, without isolation of Drug Substance. One specific batch is prepared independently for each patient.

A general flowchart has been provided that includes information regarding the process parameters/operational ranges and the control methods. Some information was provided regarding the manufacture of *E. coli* L-asparaginase used for the encapsulation in erythrocytes to obtain eryaspase.

The source of the L-Asparaginase has been changed and as no comparability results have been presented, a major objection has been raised.

The Process validation and/or evaluation section includes an evaluation of process parameters that can potentially impact the encapsulation process.

The elucidation of the structure of eryaspase consists mainly in the verification that the characteristics of the erythrocytes and of the L-asparaginase that constitute eryaspase keep their properties after the manufacturing process. The Applicant indicates that previous studies have shown that encapsulation did not affect the functional properties of L-asparaginase in the RBCs.

Finished Medicinal Product

Graspa is an individually prepared final product manufactured according to the prescription for each patient. Encapsulated erythrocytes are formulated in a preservative solution. According to the Applicant, no changes in formulation have occurred.

Stability studies were conducted and included in the characterization section for functionality of Graspa. The claimed shelf life for the finished product is still not considered sufficiently justified and thus, the Major objection remains. There are also a number of other concerns related to the ability of the osmocells method to be stability indicating. The change in L-asparaginase could also have implications for the DP stability. The Applicant should address this issue by including DP stability studies in the comparability exercise between both asparaginases.

Adventitious agents

Leukoreduced Red Blood Cells (pRBCs) and *E.coli* L-asparaginase are the only biological starting materials used for the manufacture of the eryaspase. Graspa manufacture itself is considered not to present a risk from a microbial and viral safety perspective.

3.1.4. Conclusions on the chemical, pharmaceutical and biological aspects

Four Major Objections were raised at Day 120. After the assessment of the responses, three of the four Major Objections are considered solved, and only the one concerning stability is remaining. However, due to the change in the source of L-asparaginase, a new Major Objection has been raised concerning the lack of comparability results between the previous (native) and the current (recombinant) L-asparaginase, the implications of this change, and the potential requirement for further non-clinical/clinical bridging studies.

A number of deficiencies (Other Concerns) still remain that also need to be addressed before a positive opinion on Quality can be granted.

An additional issue is that the Applicant's definition of active substance for this medicinal product as being eryaspase is not agreed. Instead, and in line with previous advice provided by the CHMP and CAT to the Applicant, the L-asparaginase has to be considered as the active substance (see New Active Substance discussion).

3.2. Non clinical aspects

3.2.1. Pharmacology

The Applicant did not carry in vitro studies with encapsulated L-ASNase encapsulate in erythrocytes due to the low stability of the product in culture. L-ASNase encapsulation does not seem to impair the its activity and it may be considered that the in vitro data from free L-ASNase can be extrapolated to L-ASNase encapsulated in erythrocytes. In vivo studies were not carried out with the final product but with a mouse surrogated as blood compatibility issued would prevent to carry out studies with the final human product. In in vivo studies it was observed that when using the mouse surrogate of L-ASNase encapsulated in erythrocytes it was seen a significant depletion of L-asparagine levels. In a study where doses from 100 to 200 UI/KG were administered through a single dose, levels reported after 15 minutes were <2 µM. This effect could be maintained for a period up to 28 days, rebounding to values close to normal after 34 days. Therefore the product could maintain for almost a month a significant L-ASNase. Other studies obtained from literature suggest that indicate that approximately L-ASNase not encapsulated (free) maintains its activity for approximately 2-3 days. Consequently this feature provides this product with the capability of reducing the frequency of administration whilst maintaining low levels of L-asparagine. It was not confirmed by the Applicant the level of L-asparagine for cancer cells to enter into apoptosis and further information was requested. The Applicant clarification indicates that sustained partial depletion is not attainable and in the studies conducted complete L-asparagine was achieved. The administration of eryaspase IV at doses of 100 and 200 IU/kg results in complete depletion of mouse plasma L-asparagine and doses of 200 IU/kg results in observable antineoplastic activity in pancreatic cancer in mice. The reported dose range is also consistent with published data for depetion of asparagine and its concomitant antineoplastic effect.

The potential effect of anti-asparaginase antibodies on the PK properties of L-ASNase encapsulated in mouse erythrocytes was also assessed. Data suggest that in mice treated with the mouse surrogate of L-ASNase encapsulated in erythrocytes the presence of antibodies do not impair or affect significantly the efficacy of the product. This data is indicative that the erythrocyte membrane prevents to some extent an immune response against bacterial-derived L-ASNase. Although this data is quite promising long term effects were not evaluated in the dossier. This may be considered acceptable as clinical evaluation is where the final confirmation of long term efficacy should be expected.

The antineoplastic activity of L-ASNase encapsulated in erythrocytes was assessed in mice bearing lymphoma cells showing promising results. Free L-ASNase efficacy was also assessed in murine leukemic models including ALL models. Efficacy of E. coli-derived L-ASNase encapsulated in erythrocytes was reported in DBA/2 mice bearing lymphoma cells (L5178Y), a higher efficacy of the encapsulated L-ASNase compared to its free form was also seen in a 6C3HED murine lymphoma tumor model. No additional in vivo assessment of the antineoplastic activity of L-ASNase encapsulated in erythrocytes was performed by the Applicant. Although accurate levels of L-asparagine were not calculated in vivo for cancer cell survival, in vivo studies suggest that the levels achieved are sufficient to achieve a significant activity and a potential higher efficacy than free L-ASNase.

With regards of secondary pharmacology L-ASNase encapsulated in erythrocytes activity also results in production of L-aspartic acid. This issue could have a deleterious effect on the activity of the product, should it accumulate inside the erythrocyte. It was observed that the produced L-aspartic acid was also released from erythrocytes into plasma (up to two fold increase detected in plasma) effect coupled

with decrease in L-asparagine levels. Significant adverse effects were not observed in any mouse included in these studies.

The Applicant has assessed safety pharmacology with data from safety pharmacology studies performed with a recombinant form of L-ASNase (r-L-ASNase). This approach has been supported by the CHMP as clinical data from conventional L-ASNase was available.

The series of studies with recombinant asparaginase evaluated the major organs systems. In vitro studies performed in guinea pig ileum suggest that concentrations of up to 50 U/mL had no spasmogenic or spasmolytic effect. A single administration of up to 10 000 U/kg i.v. has no effect on the cardiovascular system in the dog or the respiratory and central nervous systems in the rat and dog. However, in the rat, recombinant asparaginase was shown to increase the excretion of Na+, K+ and/or Cl- at ≥100 U/kg and increased urine excretion at the maximum doses tested (10,000 U/kg). The Applicant was requested to discuss how the effective doses or no-effect doses/concentrations compared to those proposed clinically and is requested to do so and ensure that this is included in SPC accordingly. The Applicant has stated that the non-clinical urinalysis data did not results in any hypovolemia in the rats with increased Na+ excretion. Nor was any hyponatremia as a result of excessive NA+ observed in preclinical in vivo studies, and doses administered far exceed the proposed clinical dose. Increased K+ and Cl- was seen in rats at clinically relevant doses, with no histopathology findings in kidney. And hence no further warnings are proposed for the SPC, especially in light of the fact that such warnings are not in place for the Spectrilia SmPC.

Bibliographic data highlights the ability of L-ASNase 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). The Applicant was requested to discuss the clinical significance of these reported findings in comparison to doses that are proposed clinically (on a U/m2 basis). In the responses it was highlighted the lower clinical doses and reduced frequency of dosing of encapsulated L-asparaginase in comparison to the doses of 'free' L-asparginase that resulted in the reported adverse effects. Subsequently the risk associated with the proposed product are expected to be minimal in comparison, however adequate warnings are included in the proposed SPC.

Pharmacodynamic drug interactions were not assessed with L-ASNase encapsulated product but with free L-ASNase and other medicines employed in ALL chemotherapy. This issue is assessed in the clinical AR in detail. No additional pharmacodynamic drug interactions with the encapsulated L-ASNase compared to the free enzyme. Consequently the Applicant did not carry out additional drug interaction studies.

3.2.2. Pharmacokinetics

Although significant technical constraints of the animals models used has been seen, those are acknowledged and the use of mice erythrocytes together with L-ASNase as a surrogate is agreed despite its limitations.

The product is to be administered IV in the clinical practice. The half-life of L-ASNase encapsulated in mouse erythrocytes was reported range from 8.38 days to 8.55 days when administered at a dose of 100 to 200 IU/kg. This half life extension of L-ASNase is considerably higher than the obtained from free L-ASNase in humans which has been described to achieve values that range between 14 to 22 hours while in mice free L-ASNase values have been described to achieve half lives ranging from 2.4 to 5 hours. The encapsulation process do not seem to affect significantly to the erythrocyte half life although no data has been forwarded regarding the effects on the oxygen transportation capabilities.

Half lives prolongation of L-ASNase were also described in other species as dogs and monkeys apart from mice and humans. The prolonged half life of L-ASNase encapsulated in erythrocytes can be due to the barrier that the erythrocyte membrane displays protecting L-ASNase from the standard pathways that free L-ASNase is exposed to and instead following a the same degradation process than the erythrocytes in which they are encapsulated.

No distribution studies have been performed by the Applicant in compliance with CHMP scientific advice received. No differential distribution of erythrocytes is expected. Consequently L-ASNase distribution is expected to follow the erythrocytes in which they are encapsulated. Although free L-ASNase may cross the placenta, encapsulated L-ASNase is not expected to do so as erythrocytes do not normally cross the placental barrier.

L-ASNase is encapsulated in erythrocytes and expected to be degraded following the same process as erythrocytes consequently no metabolism studies were deemed necessary.

No studies have been carried out to evaluate the excretion of the product. No excretion studies are deemed necessary for erythrocytes and considering that L-ASNase is a protein no differential excretion is deemed compared to other proteinic molecules.

The Applicant bases the lack of pharmacokinetic drug interactions on the available data of free L-ASNase together with the extrapolability this data to the erythrocyte encapsulated product. The Applicant's justification is considered acceptable.

3.2.3. Toxicology

Single dose administration was assessed unintendedly in a repeated dose toxicity study in mice which had to be terminated prematurely due to unexpected toxicity following the second dose. The only adverse finding reported following single dosing was perivenous inflammatory cell infiltrate at the site of injection of the surrogate encapsulated product.

Adverse effects in published data from free E. coli L-ASNase administration were not seen in the Applicant's studies in which animals were dosed L-ASNase encapsulated in erythrocytes. Adverse effects seen in published data in monkeys dosed with L-ASNase erythrocytes encapsulated resulted in increase in serum amylase and lipase levels observed at the highest dose and Anti-asparaginase IgG antibodies but only in animals receiving the L-ASNase encapsulated but not in animals receiving free L-ASNase. Among the main findings described following administration in various animal species dosed with non encapsulated L-ASNase it can be highlighted the following: in hyperglycemia, hypolipoproteinemia, hypoalbuminemia, coagulation factor deficiencies, hepatotoxicity and pancreatitis. In rabbits, the most sensitive species findings included Hypoparathyroidism with tetanic symptoms, hypocalcemia, hypomagnesemia, and, in most of animals, hyperphosphatemia resulting in death.

The product under evaluation is composed of two main parts. On one side the erythrocyte and on the other side the L-ASNase which is encapsulated into the erythrocyte.

Due to blood compatibility issues the human product could not be administered to animal models. A mouse surrogate using mouse erythrocytes was then selected for the toxicology evaluation together with data with free L-ASNase from animal models and human patients. The L-ASNase encapsulated in mouse erythrocytes was maintained in bovine serum albumin (BSA) for stability.

As mentioned before the repeated dose study could not be completed and was terminated prematurely. The highest technically feasible dose level (200 IU/kg) was administered by the IV route at 14 day-intervals based on the calculated half-life of the mouse product. Immediately after receiving the second dose animals displayed severe clinical signs included death. Those effects were attributed

to an anaphylactic reaction triggered BSA contained in the vehicle of the product. Several adverse events were seen only in males although the Applicant could not identify the reason behind this differential toxicity although as the human product does not contain BSA related adverse events are not expected in humans. Adverse findings were also seen in vehicle administered mice (SAG mannitol + 6% BSA). No relevant and translatable data is available from repeated dose administration. Attempts to substitute BSA with another preservative in order to circumvent the adverse finding reported which could produce reliable data were unsuccessful. In scientific advice the CHMP agreed to not perform another repeated dose toxicity study. Nonetheless, it is noted that among 62 mice including 51 males and 11 females that received 2 injections of L-ASNase encapsulated in mouse erythrocytes or the vehicle on both day +1 and day +15, lethality was noted predominantly in males (3/38 in the vehicle group, and 2/13 in the group treated with L-ASNase encapsulated in mouse erythrocytes) on day +15 (i.e. shortly after the second administration) and no females died or even developed adverse clinical signs after the second dose of L-ASNase encapsulated in mouse erythrocytes. The Applicant was requested to discuss the plausible reasons for this taking into account any relevant toxicokinetic data (i.e: anti-asparaginase antibody titers and asparaginase serum activity). The Applicant clarified satisfactorily this issue explaining that that the observed mortalities in males were likely related to vehicle and not to the test product. The low numbers involved were not considered significant to attribute sex differences especially as in animals treated twice with the murine surrogate, 5/51 males died versus all 11 females surviving. It is agreed that based on the higher proportion of males administered treatment no further discussion is warranted, especially as there were no monitored parameters linked to the sex of the animals. Furthermore asparaginase activity was consistent amongst both sexes.

CHMP advice received in 2012 [EMEA/H/SA/991/2/2012/PA/ADT/SME/I] discussed the fact that the applicant intended to administer gemcitabine together with Graspa in the clinic. Therefore a mouse study was proposed to investigate the potential toxicological interactions between gemcitabine and Graspa, focusing on the organs known to be target of gemcitabine toxicity (lung, bone marrow, kidney). The applicant has not presented any data regarding the co-administration of Graspa with gemcitabine. The applicant was requested to justify the absence of these data. In the Applicant's response it was provided study reports where eryaspase and gemcitabine were co-administered in mice (studies R-EGG0-2-61 and R-EGG0-2-64. In the first study, it was shown that mGraspa® had no significant impact on gemcitabine's metabolism and depletion of asparagine was effective for all the time of study. No exacerbations of known toxicities, or new toxicities were noted and no variation in the haematology and blood biochemistry parameters were considered connected to mGraspa® treatment. However, the occurrence of three deaths (out of 60 mice) in the same group receiving mGraspa®+GEM remain unexplained and the report states that this would require further investigations to definitively conclude on the potential toxicity of the drugs given in combination. The second study was conducted because in study R-EGG0-2-61 lungs and bone marrow (targets of gemcitabine toxicity) were not included in the list of organs to be specifically analysed for toxicity. Hence the second safety pharmacology study was performed using the same sequence and doses of administrations to specifically test the effects of the drug combination on lungs and bone marrow. Evidence of plasma levels of the test items were comparable to that obtained in the first study. Cytology on bone marrow didn't evidence abnormal cell counts or cell maturation. No clinically significant abnormal morphologic findings were recorded. And although lower lung weights were observed in the combination group – this was not statistically significant and hence it was concluded that the combination treatment did not exacerbate the known gemcitabine toxicities. Taken together, from a non-clinical perspective, it is agreed that co-administration of eryaspase and gemcitabine is not expected to exacerbate the known toxicities for the individual test items.

The applicant presented resultsthat describes the effects of repeated administration of the 'free' L-ASNase to be encapsulated to form eryaspase. Ten animals (rat)/group/sex were given 100, 1,000 or 10,000 IU/kg/day of lyophilized AsparaginaseNo premature mortality was noted and the NOAEL was determined to be below 100 IU/kg of Asparaginase 10,000 medac after repeated i.v. administrations. A number of findings were noted and effects on the kidney (changes in urinalysis and kidney weight) and effects on haematological parameters were still apparent following the recovery period at all doses studied. It is noted that daily dosing during the 28 day study is more frequent than that proposed clinically (i.e. dosing every three weeks). The applicant was therefore requested to present how these findings relate to the expected clinical exposure levels for Graspa. The Applicant stated that effects on urinary parameters and kidney weights were only monitored in high-dose animals after the recovery period and no 'statistically significant' test item related findings were noted. Furthermore significant effects following urinalysis were not observed in low dose animals (100 IU/kg) – hence the reported NOAEL. However although effects on haematological parameters were still apparent after the recovery period (high-dose animals only; this followed dosing with free L-asparaginase at 10,000 IU/kg, daily, which is far higher than eryaspase proposed clinical doses (100-200 IU/kg, weekly to monthly). Taking into account the difference in dosing schedule and the moderate effects noted in the low dose animals it is accepted that a low potential for toxicity can be expected. This is supported by the findings reported for a similar study (LPT 16367/1/02) with recombinant L-asparaginase which suggests a similar safety profile for asparagine 10.000 medac versus Spectrila as encapsulated L-asparaginase

Most publically available information included mainly data from L-ASNase from E. coli. Animal models included rats, dogs and Rhesus monkeys. Adverse events reported included weight loss, decreased food consumption, nausea, diarrhea, hypersensitivity reactions, fatty liver, pancreatitis, gastrointestinal toxicity, anemia, hypoparathyroidism and thymus enlargement. Some animals displayed antibody response against L-ASNase. Liver and pancreatic toxicities were generally reported. Liver toxicity has been related to the reversible inhibition of protein synthesis by L-ASNase and in pancreas may be a result from insulin synthesis interference (low levels of insulin precursors). Modified levels of fibrinogen and clotting factors, with concomitant in prothrombin and partial thromboplastin time increases were seen. Clinical findings confirmed of thrombosis, bleeding or hemorrhage have been described. In rabbits also L-ASNase administration resulted in parathyroid alterations and hypersensitivity reactions the latter also reported in dogs. Liver toxicity was also clearly seen in monkeys where abnormal liver function was seen. Fatty infiltration of the liver was associated with alterations in serum parameters of liver function.

Reported adverse effects using free L-ASNase do not necessarily reflect those that could be potentially reported when using the encapsulated L-ASNase product as the erythrocyte prevents close contact of the enzyme with organs and tissues although similar events in humans cannot be ruled out.

Classic genotoxicity studies are not warranted for Eryaspase as L-ASNase encapsulated in erythrocytes. The genotoxicity data provided regarding L-ASNase, although it was obtained from publically available sources lacking of GLP compliance, is considered relevant for the evaluation. Data provided do not suggest that L-ASNase has relevant genotoxicity potential.

No classic carcinogenicity studies have been provided by the Applicant. Due to the nature of the product and bearing in mind the lack of potential genotoxicity/carcinogenicity signals from L-ASNase and the fact that there is a substantial clinical supportive data from L-ASNase administration, the lack of carcinogenicity studies is considered acceptable taking also into account current ICH S6 guidance ICH S6.

No data regarding the effects of L-ASNase have been provided. Although fertility studies is not warranted to support marketing of pharmaceuticals intended for the treatment of patients with

advanced cancer the Applicant was requested to provide information regarding the product's effect on reproductive organs as the basis of the assessment of potential impairment of fertility or alternatively provide adequate justification employing clinical data available from enzyme (L-ASNase) administration. The Applicants response indicates that since the clinical experience with L-asparaginase is long and well documented without relevant effects on fertility and since the requested histopathology data on reproductive organs do not reveal discernible signs of toxicity, the issue may be considered as solved.

No studies on embryo-fœtal development have been carried out by the Applicant. Public scientific data provided evidences the teratogenic potential of L-ASNase in several animal models. Findings included malformations in the CNS (spina bifida, hydrocephalus), heart and skeleton anomalies, gastroschisis, and missing tail, in the offspring of mice, rats, rabbits and chicken. No differential toxicity is expected from the L-ASNase encapsulated in erythrocytes. The SmPC reflects that the product should not be used during pregnancy.

Bearing in mind ICH S9 guideline, the product nature, the indication of the product and that Asparaginase 10,000 medac has been approved in several countries for the treatment of ALL, the lack of studies that evaluate prenatal and postnatal development including maternal function is accepted.

The PDCO did not request additional studies to evaluate the product in juvenile animals. Taking into account that there is a lot of clinical experience with Asparaginase 10,000 medac and that its encapsulation it is not expected to result in new concerns for the paediatric population, the lack of such studies is considered acceptable.

Local tolerance has been evaluated within the toxicity studies performed. The only finding reported was slight deposit of perivenous fibrin with mononuclear cells in the administration site when L-ASNase encapsulated in mouse erythrocytes was dosed to mice. No other relevant findings have been reported due to the administration of the product.

Results obtained in guinea pigs which received a rabbit serum containing high titers of anti-asparaginase antibodies are indicative that the response against the administered product is much reduced to L-ASNase encapsulated in erythrocytes than those reported when the same dose of free L-ASNase was administered in this animal model. Data is suggestive the erythrocyte creates a barrier that protects the L-ASNase from direct interaction thus reducing the severity of the adverse events.

The product immunogenicity has not been assessed in dedicated studies. Public available literature indicates that L-ASNase administration in preclinical studies result in decreased humoral and cellular immune responses. Those findings were seen in mice at doses from 200 IU/kg/day of L-ASNase (IP). Immunosuppressive effects appear to decrease over time. In mice decrease in thymus weight and cellularity and in spleen weight with changes in the spleen B cell germinal areas was reported for L-ASNase from E. coli origin. In vitro L-ASNase lessened proliferative response of lymphocytes to mitogens this effect was partially modulated by the addition of L-asparagine or L-glutamine to the cultures. Immunosuppressive effect was sufficient to prolong graft survival in mice. Encapsulated L-ASNase is not foreseen to result in additional adverse effects to the administration of L-ASNase alone. Immunosuppressive effects of L-ASNase are expected to be modulated by its encapsulation in erythrocytes and due to the reduced glutaminase activity once the enzyme is encapsulated.

Glutamine depletion may be associated with hepatotoxicity. Studies revealed that E. coli L-ASNase administration resulted in liver toxicity in mice and humans. Similar results were seen for *Erwinia carotovora* L-ASNase nonetheless no hepatotoxicity was reported when free L-ASNase from *Vibrio succinogenes* was studied maybe due to the low activity of the enzyme for L-Glutamine [130 to 600 fold less (Disasio et al 1976)] whilst maintaining its anti-lymphoma activity. The Applicant did not

explain why L-ASNase from E.Coli was selected for the product instead the L-ASNase from *Vibrio succinogenes* taking into account the more favourable safety profile of the latter. Considering the above information the Applicant was requested to clarify the reason behind selecting L-ASNase from E.Coli for the product development instead of another enzyme with a better safety profile. In brief, the Applicant indicated in the response that despite there was a more favourable safety profile of other L-asparaginase, no information was available from a clinical perspective and since no there was no GMP product available for the potential alternative candidates, unfortunately they were not considered as candidates for the development of the product.

3.3. Clinical aspects

• *Tabular overview of clinical studies*

Table 1-1 Clinical pharmacology studies with GRASPA

Study Number	Type of study/population	Number of patients	Duration
GRASPALL 2005-01 (20 May 2011)	Phase I/II, comparative, randomized, parallel group study of L- asparaginase or GRASPA 50, 100 or 150 IU/kg in adults and children aged ≥1 year, with ALL, in combination with multi-agent chemotherapy	N= 24: 12 children, 12 adults	8 weeks treatment + 12 month follow-up
GRASPALL/GRAALL SA2-2008 (27 Sep 2013)	Phase IIA, open-label, dose escalating study of GRASPA 50, 100, or 150 IU/kg in Philadelphia chromosome-negative (ALL Ph-) adults aged ≥55 years with ALL in combination with multi-agent chemotherapy	N=30	22 to 28 days treatment in induction 24 month follow-up
GRASPALL 2009-06	Phase II/III, comparative, randomized, parallel-group study of L-asparaginase or GRASPA 150 IU/kg in adults and children (1-55 years) with ALL in first relapse, in combination with multi-agent chemotherapy	N=80: 57 children, 23 adults	24 weeks treatment + 36 month follow-up
GRASPANC 2008-02	Phase I, dose escalation study of GRASPA 25, 50, 100 or 150 IU/kg in patients aged between 18 and ≤70 years with pancreatic cancer	N=12	Single treatment + 56 days follow-up

[a]Four patients received an unplanned 120 IU/kg intermediate dose instead of 150 IU/kg, and 1 patient received 100 IU/kg instead of 150 IU/kg. These patients with high body weight received a lower proportion of the prescribed dose due to the limitations on volume of transfusion.

3.3.1. Pharmacokinetics

The PK, pharmacodynamics (PD), and immunogenicity of Graspa were assessed in 3 clinical studies in patients with ALL that included 62 adults (32 adults aged 18-55 years and 30 adults aged ≥55 years) and 66 children. The PK data from a study of Graspa in 12 adult patients with pancreatic cancer is also included in this module as supportive information.

No PK studies were performed in healthy subjects, patients with renal or hepatic impairment, patients who were pregnant or breast-feeding. The PK analysis included an assessment of the effects of age and gender on encapsulated L-asparaginase activity. No specific interaction studies have been performed with Graspa. The relationship between Graspa dose levels and response was not formally performed due to limited number of patients in the early Graspa clinical studies.

Parameters used to assess PK, PD and immunogenicity in each study are summarized in Table 1-2.

Table 1-2 Pharmacokinetic, pharmacodynamic and immunogenicity parameters by study

Parameter	GRASPALL 2005-01	GRASPALL/GRAALL SA2-2008	GRASPALL 2009-06	GRASPANC 2008-02
Pharmacokinetics				
Encapsulated L-asparaginase activity [Difference between total[a] and free L-asparaginase]	+	+	+	+
Free L-asparaginase activity (plasma)	control		control	
Amino acids concentration vs. time				
Plasma amino acids (asparagine, aspartic acid, glutamine, glutamic acid)	+	+	+	+
CSF amino acids (asparagine, aspartic acid, glutamine, glutamic acid)		+		
Pharmacodynamics				
Plasma asparagine depletion <2 µm/L after first administration	+	+	+	+
Duration of asparagine depletion after second injection	+	+		
Asparagine depletion in CSF	+	+		
Immunogenicity				
Antibody status (*E. Coli* anti-asparaginase antibodies)	+	+	+	+

a Total encapsulated L-asparaginase activity in whole blood was measured as part of the method to determine encapsulated L-ASNase activity in the RBC. These data are not analyzed separately.

Absorption

- **Bioavailability**

The product is administered by intravenous infusion.

Table 2-13 Encapsulated L-asparaginase PK Parameters Following First Infusion

Treatment	Statistics	Cmax (IU/L)	AUClast (day*IU/L)
Allergic adults – GRASPA	N	11	11
	Mean	1178.404	13570.426
	SD	594.331	7434.157
	Min	384.36	6420.00
	Median	939.39	10251.14
	Max	2290.36	30462.13
	CV%	50.4	54.8
	Geom. Mean	1046.842	12097.293
	CV% Geom. Mean	55.92	51.33
Allergic children-GRASPA	N	15	15
	Mean	1872.554	14625.420
	SD	790.293	6297.830
	Min	732.46	5540.19
	Median	1797.76	14003.14
	Max	3507.48	26244.47
	CV%	42.2	43.1
	Geom. Mean	1713.454	13348.081
	CV% Geom. Mean	47.08	47.74
Non-allergic adults-GRASPA	N	5	5
	Mean	1478.426	14692.124
	SD	517.731	5069.979
	Min	959.48	7060.64
	Median	1488.34	15826.38
	Max	2257.32	20667.75
	CV%	35.0	34.5
	Geom. Mean	1409.389	13839.974
	CV% Geom. Mean	35.46	42.87
Non-allergic children – GRASPA	N	21	21
	Mean	1535.399	13553.930
	SD	423.964	6388.340
	Min	583.85	6081.93
	Median	1479.61	11288.39
	Max	2466.04	33951.13
	CV%	27.6	47.1
	Geom. Mean	1474.346	12460.864
	CV% Geom. Mean	31.27	41.95

Source: Addendum Report GRASPALL 2009-06 in Module 5.3.5.1.

- **Influence of food**

No studies have been carried out to investigate the effect of food on the pharmacokinetic profile of Graspa, as it is not considered relevant for a product that is administered by intravenous infusion.

Distribution

Graspa remains in the intravascular space.

Elimination

Half-life of Graspa based on encapsulated asparaginase activity

Calculation of the half-life of Graspa has been performed firstly using the half-life of the encapsulated asparaginase activity.

Half-life has been calculated in two ways considering:

- All injections of Graspa - 28 in total including 18 initial injections and 10 re-injections.

  Or

- Only the 18 initial injections to reduce potential bias linked to a potential "second injection effect" as well as" patient effect".

The summary analysis of half-life of Graspa taking into account only the 18 initial injections is presented below. No major difference is observed as compared to the calculation base on all 28 injections.

Table 16 : Half-life of GRASPA® (in days) taking account the 18 initial injections of asparaginase

	GRASPA 50	GRASPA 100	GRASPA 150	All
Mean	20.85	37.24	63.30	40.46
S.E.	4.54	9.38	33.59	11.80
Median	23.25	35.60	27.49	23.25
Minimum	5.94	14.04	10.84	5.94
Maximum	37.27	68.88	222.61	222.61

Half-life of Graspa based on administered processed red blood cells

Calculation of the half-life of Graspa has been performed secondly using the survival of processed RBC constituting Graspa.

Calculation of half-life has been done in a similar way as asparaginase encapsulated in Graspa®, i.e. considering either all injections of Graspa (19 injections in total corresponding to the patients with a D positive blood group and transfused with a D negative RBC encapsulating asparaginase bag) or considering only the initial injections.

Graspa group : Samples were collected 15 minutes, 6 hours after administration and then at day 1, 12 and 24 of FI/F2 blocks and at day 40, 60, 90 and 120 after administration.

Half-life of RBC of Graspa for all injections is presented in the table below. Mean half-life was 26 days and no statistical difference was observed between the 3 doses of Graspa. The summary analysis of half-life of processed RBC of Graspa taking into account only the initial injections is presented below. No major difference is observed compared to the calculation base on all injections.

Table 18 : Half-life of processed RBC of GRASPA® (in days) - initial injection

	Graspa 50	Graspa 100	Graspa 150	All
Mean	18.81	13.81	37.75	24.33
S.E.	7.26	1.34	12.77	5.86
Median	15.97	12.98	36.98	16.44
Minimum	5.72	12.01	13.40	5.72
Maximum	37.56	16.44	63.63	63.63

- **Metabolism**

No clinical studies were designed to establish the distribution, metabolism or excretion of Graspa. Due to the protein nature of L-asparaginase encapsulated in erythrocytes, no metabolism or excretion studies were conducted.

- **Dose proportionality and time dependency**

Overall, mean Cmax and AUC_{inf} of encapsulated L-asparaginase increased with increasing doses from 50 to 150 IU/kg for the adults and children. However, the increase did not seem to be dose proportional. A statistical assessment of dose proportionality was not conducted.

Mean t½ of encapsulated asparaginase ranged from 14-18 days for the adults and children. The PK parameters following the first and second infusion in study GraspaLL 2005-01 seem to be similar for the first and second infusion of 150 IU/Kg, although there was not complete washout of L-asparaginase between the first and second infusion (Table 2-2).

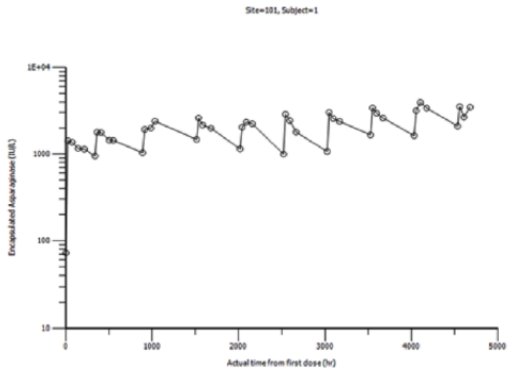
Table 2-2 Encapsulated L-asparaginase PK Parameters by Dose and Infusion

Treatment (IU/kg)	Infusion	Statistic	t½ (day)	Cmax (IU/L)	AUClast (day*IU/L)	AUCinf (day*IU/L)	Tlast (day)
50	1	N	4	6	6	4	6
		Mean	15.39	973.33	21310.98	12382.64	56.70
		SD	4.73	544.01	19602.36	10194.32	25.83
		Min	8.89	270.00	5340.83	5782.34	29.93
		Median	16.37	885.00	13088.09	8080.38	51.50
		Max	19.93	1830.00	50404.87	27587.46	89.11
50	2	N	2	3	3	2	3
		Mean	28.14	473.33	9936.26	11265.07	74.47
		SD	3.60	225.46	1481.69	839.15	13.58
		Min	25.59	240.00	8901.23	10671.71	58.97
		Median	28.14	490.00	9273.97	11265.07	80.18
		Max	30.68	690.00	11633.59	11858.44	84.27
100	1	N	5	6	6	5	6
		Mean	15.93	1208.33	12122.38	16692.84	43.18
		SD	4.19	290.27	4996.27	4081.81	38.63
		Min	8.61	700.00	6072.98	10900.12	11.99
		Median	17.74	1320.00	12086.59	18461.46	33.00
		Max	18.63	1460.00	20701.32	21213.08	120.11
100	2	N	3	4	4	3	4
		Mean	19.12	880.00	15172.12	12968.81	62.28
		SD	9.05	484.63	9168.62	8172.59	39.23
		Min	8.72	290.00	3764.52	4518.91	20.03
		Median	23.53	935.00	15971.11	13555.02	61.52
		Max	25.13	1360.00	24981.72	20832.51	106.04
150	1	N	6	6	6	6	6
		Mean	17.80	2133.33	27008.30	32208.01	68.97
		SD	6.39	792.48	12817.71	9715.72	44.21
		Min	13.34	950.00	12212.89	23664.24	27.00
		Median	14.23	2070.00	26114.17	29612.58	57.37
		Max	28.27	3070.00	49509.63	51180.28	122.27
150	2	N	3	3	3	3	3
		Mean	15.39	2720.00	48091.82	63962.35	65.09
		SD	3.16	1021.22	28755.51	43934.35	31.22
		Min	13.10	1770.00	23947.76	36275.99	29.21
		Median	14.07	2590.00	40423.59	40990.78	80.03
		Max	18.99	3800.00	79904.10	114620.28	86.04

Source: Addendum Report GRASPALL 2005-01 in Module 5.3.5.1.

Several patients in the pivotal Study GraspaLL 2009-06 received multiple infusions. Following the first infusion, the PK profile was consistent with near steady state conditions, Figure 2-2.

Figure 2-2 Encapsulated asparaginase concentration profile for Patient 101-1 (Source: Addendum Report GRASPALL 2009-06 in Module 5.3.5.1)



Source: Addendum Report GRASPALL 2009-06 in Module 5.3.5.1.

Intra- and inter-individual variability

In study GraspaLL 2005-01, the %CV for AUCinf in the 50 IU/kg (82.3%, n=4) group was higher than for the 100 IU/kg (24.4%, n=5) and 150 IU/kg (30.2%, n=6) dose groups.

In pivotal study GraspaLL 2009-06, following the first infusion, the %CV for C_{max} ranged from 27.6 to 50.4% and for AUC_{last} from 34.5 to 54.8% n=4). %CV is higher across all doses (C_{max}: 34.7-63.0; AUC_{last} total: 73.6-128.9; T_{last}: 78.5-111.53).

The inter-individual variability seems to be moderate-high. However, the number of patients is limited and should be considered with caution. Intra-variability has not been analysed.

Pharmacokinetic in target population

No PK studies were performed in healthy subjects. Results of the study conducted in patients with locally advanced metastatic pancreatic adenocarcinoma, who failed one or two chemotherapy lines in the metastatic setting seem to be consistent with results of studies conducted in patients with ALL. Number of patients with locally advanced metastatic pancreatic adenocarcinoma is limited and results should be considered with caution.

Special populations

- **Impaired renal function**

The pharmacokinetics of Graspa was not specifically evaluated in patients with renal impairment. However, as asparaginase is a protein, it is not excreted renally, and no dose adjustment is considered necessary in patients with renal impairment. This information has been reflected in the SmPC.

- **Impaired hepatic function**

The pharmacokinetics of Graspa was not specifically evaluated in patients with hepatic impairment. Graspa is contraindicated in patients with severe hepatic impairment. Additionally, liver laboratory values such as transaminases and bilirubin levels should be monitored before and regularly throughout therapy. If patients develop severe liver impairment, treatment with Graspa should be interrupted. This information is reflected in the SmPC. However, it should be clarified under section 4.2 that no dose-adjustments are recommended in patients with mild or moderate hepatic impairment.

- **Gender**

There was no apparent difference in the PK of L-ASNase between males and females.

Figure 4 eryaspase Terminal t½ for Male and Female Patients following First Infusion

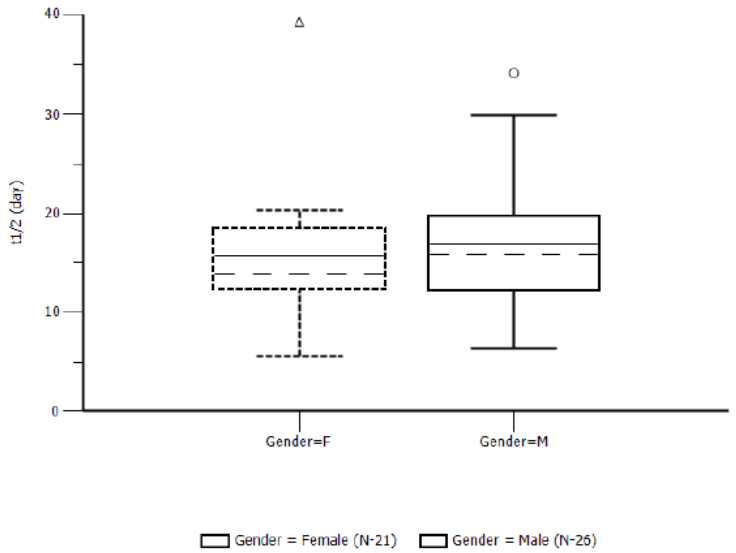


Figure 5 eryaspase Dose-Normalized Cmax for Male and Female Patients following First Infusion

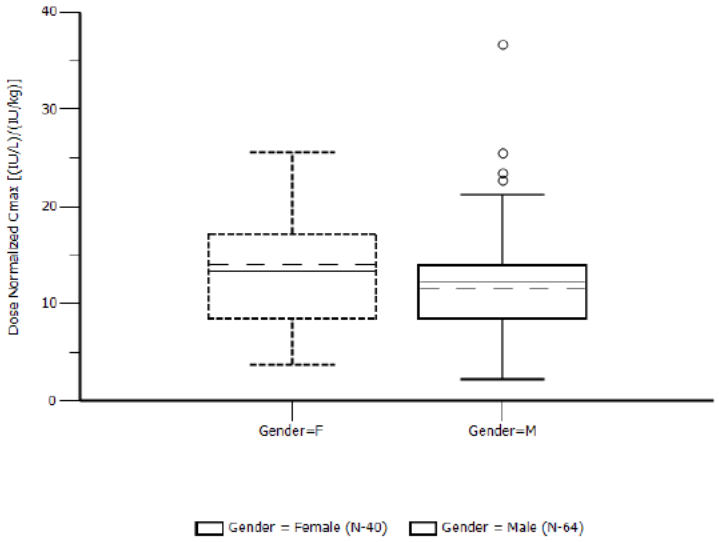
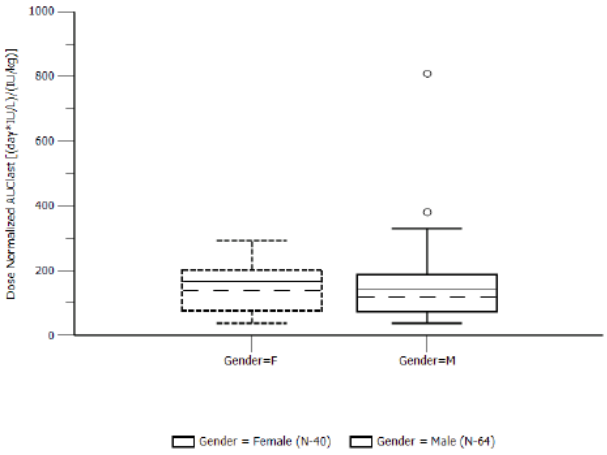


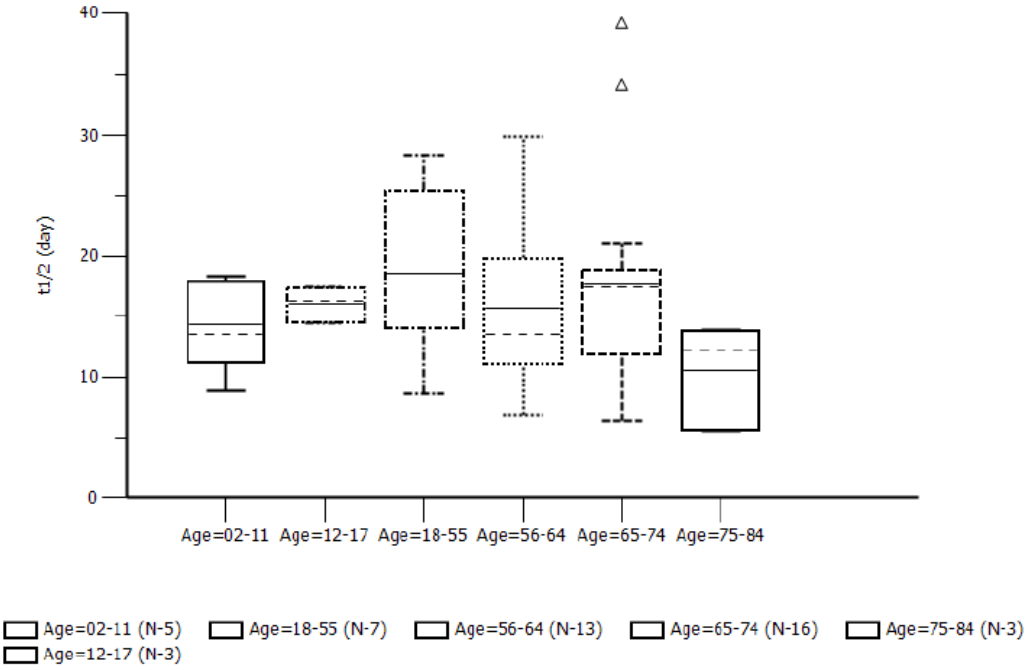
Figure 6 Eryaspase Dose-Normalized AUClast for Male and Female Patients following First Infusion



- **Age**

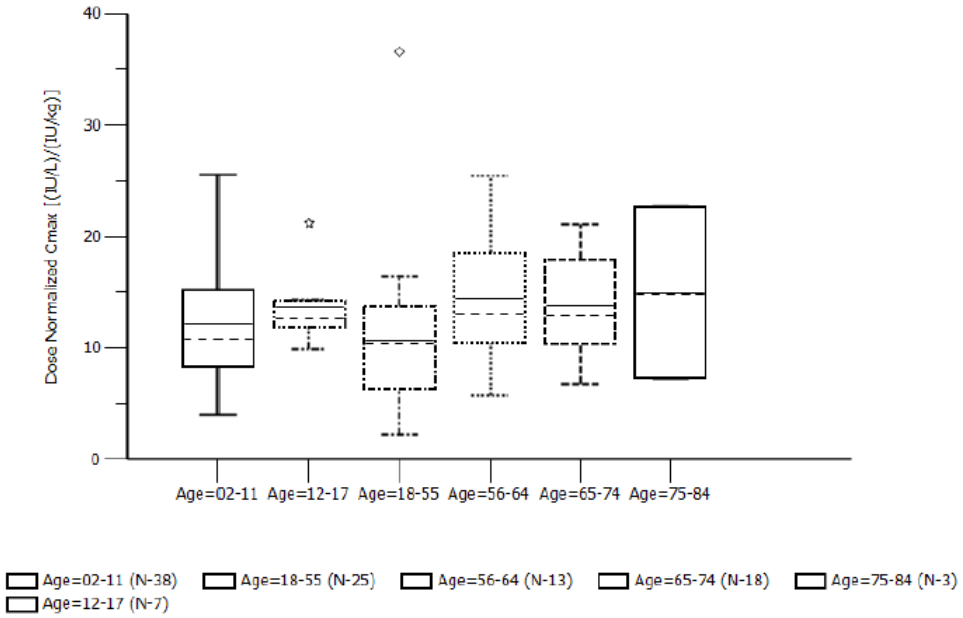
Patients were categorised into 6 age groups: 2-11 years, 12-17 years, 18-55 years, 56-64 years, 65-74 years and 75-84 years. As all patients received at least one infusion of Graspa, PK parameters following the first infusion were combined for each age category. Figure 1 shows the terminal t½ for the age categories. As a result of the short sampling duration following the first infusion in Study GraspaLL 2009-06, patients in this study did not contribute to the estimates of terminal t½. Visual inspection shows there is a consistent terminal t½ with overlap in 25-75th percentiles across the age groups.

Figure 1 eryaspase Terminal t½ for Patients following First Infusion



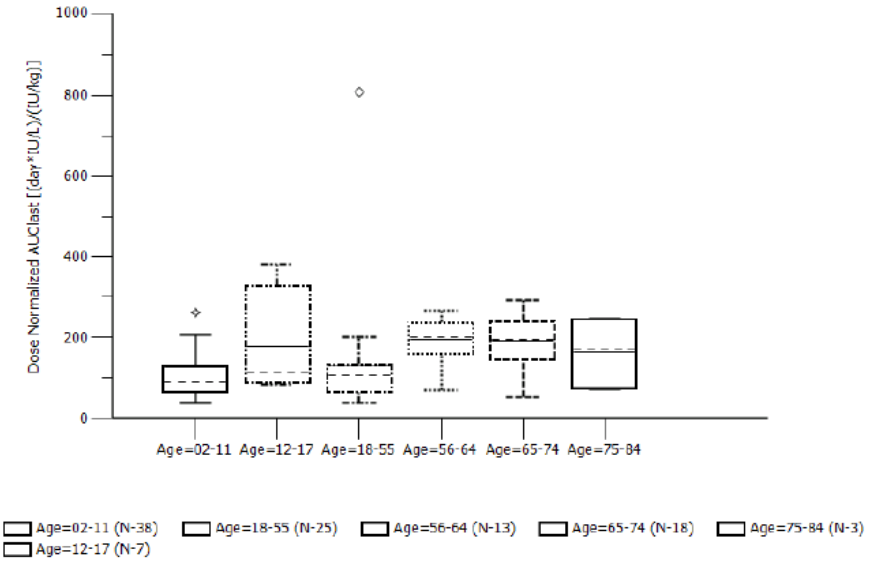
To compare C_{max} values across all dose groups, the C_{max} values were normalised by dose for individual patients in each age category. Figure 2 shows the dose-normalised C_{max} following the first infusion across the age categories. There was overlap of the 25-75th percentiles across all age groups, indicating no apparent difference in C_{max} of encapsulated asparaginase for patients aged 2 to 84yrs.

Figure 2 eryaspase Dose-Normalized Cmax for Patients following First Infusion



Similar to C_{max}, the AUC_{last} values following the first infusion were normalised by dose for patients in each age category. Figure 3 shows the dose-normalised AUC_{last} across the age categories. There was overlap of the 25-75th percentiles across all age groups, indicating no apparent difference in AUC_{last} of encapsulated asparaginase for patients aged 2 to 84yrs.

Figure 3 eryaspase Dose-Normalized AUClast for Patients following First Infusion



Data for patients above 65 years old is limited; this information is reflected in the SmPC.

The following tables reflect the treated patients by categories of age and type of cancers:

Clinical studies in Acute Lymphoblastic Leukaemia:

GRASPA treatment	Age up to 64 (Older subjects number /total number)	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
GRASPALL2009-06	52/52	0/52	0/52	0/52
GRASPALL2005-01	18/18	0/18	0/18	0/18
GRAALLSA2-2008	12/30	15/30	3/30	0/30

Clinical study in pancreatic cancer

GRASPA treatment	Age up to 64 (Older subjects number /total number)	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
GRASPANC2008-02	8/12	4/12	0/12	0/12

Interactions

- **In vivo**

Interactions with other medicinal products are those related to the use of L-asparaginase, and those related to blood transfusions. No specific interaction PK studies have been performed. However, medications with the potential to cause haemolysis were prohibited due to the potential to interfere with Graspa.

3.3.2 Pharmacodynamics

Mechanism of action

L-asparagine is a high molecular weight enzyme obtained commercially from Escherichia coli (E.coli) or Erwinia chrysanthemi composed of four identical subunits (tetramer) with one active site per subunit. It has a molecular weight of 138,368 Da. L-asparaginase hydrolyses extracellular asparagine, which is required for cell survival, to aspartic acid and ammonia. Normal cells are capable of synthesising their own asparagine but certain malignant cells (especially lymphoblastic leukaemia cells) are not. These cells therefore obtain asparagine, essential for their survival, from the extracellular environment. As L-asparaginase depletes extracellular asparagine levels, growth of lymphoblastic tumour cells is consequently inhibited.

Primary pharmacology

Results from GraspaLL 2005-01 STUDY (This Study is presented in more detail in the Efficacy section as it is the basis for the dose selection)

Duration of asparagine depletion after the first injection

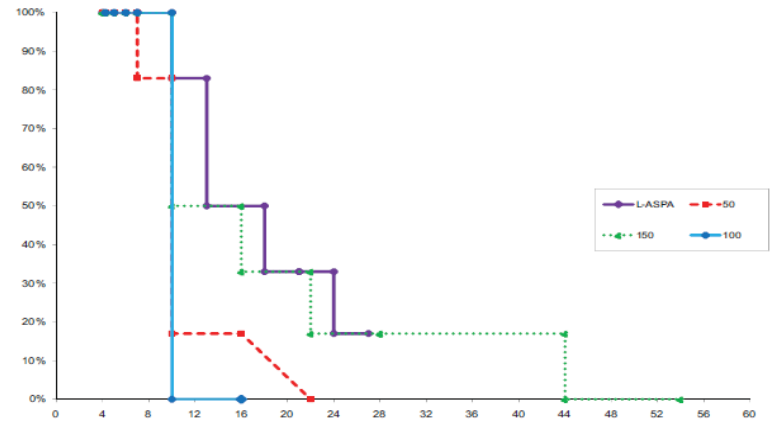
Table 7: Duration of Asparagine depletion after the first injection of GRASPA® / L-ASPA

Duration of depletion (days)	L-ASPA (N=6)	GRASPA 50 (N=6)	GRASPA 100 (N=6)	GRASPA 150 (N=6)
Minimum duration Last point value <2µmol/L	14.88 (6; 27.2)	5.97 (2.9; 12.0)	6.13 (5.9; 6.9)	14.65 (5.8; 40.0)
Maximum duration First point value >2µmol/L	24.42 (9; 48.3)	11.30 (5.8; 19.2)	11.93 (11.0; 12.9)	22.48 (12.0; 51.0)
Estimated duration **Median** (Min – Max)	20.65 18.50 (7.5, 37.8)	8.63 8.95 (4.4; 15.6)	9.03 9.00 (8.5; 9,5)	18.57 12.53 (8.9, 45.5)

For all doses of Graspa large variability in the duration of depletion obtained was noticed with for example an estimated mean of depletion duration ranging from 8.9 to 45.5 days in the Graspa 150 IU/kg group. The median duration of depletion was about 9 days, in children as well as in adults (Table 2.8).

Proportion of patients presenting asparagine depletion over time

depletion at	L-ASPA		GRASPA 50		GRASPA 100		GRASPA 150		All patients	
	N	%	N	%	N	%	N	%	N	%
3 days	6	100.0%	6	100.0%	6	100.0%	6	100.0%	24	100.0%
6 days	6	100.0%	5	83.3%	6	100.0%	6	100.0%	23	95.8%
12 days	5	83.3%	3	50.0%	3	50.0%	6	100.0%	17	70.8%
18 days	4	66.7%	1	16.7%	0	0.0%	3	50.0%	8	33.3%
24 days	2	33.3%	0	0.0%	0	0.0%	2	33.3%	4	16.7%
40 days	2	33.3%	0	0.0%	0	0.0%	1	16.7%	3	12.5%
50 days	0	0.0%	0	0.0%	0	0.0%	1	16.7%	1	4.2%



Duration of asparagine depletion after the second injection

Table 9 : Duration of asparagine depletion after second transfusion

Mean (Min ; Max)	GRASPA 50 (n=3)	GRASPA 100 (n=4)*	GRASPA 150 (n=3)
Minimum period of depletion (days) Last point with value <2	3.73 (2.20; 6.00)	1.08 (0.00; 4.10)	17.70 (6.00; 30.10)
Maximal period of depletion (days) first point with value >2	13 (10.2; 14.90)	3.5 (0.2 ; 13.20)	30.20 (15.20; 46.10)
Mean duration of Depletion (days)	6.6 (2.50 ; 6.8)	1.23 (0.00; 4.60)	18.17 (6.60; 30.60)

* NB: In GRASPA 100 group, one patient in this group received only an half of the dose, because he presented an elevation of the pancreatic enzyme before the transfusion.

Three patients (all in the GRASPA 100 IU/kg group) presented only minimal depletion duration (a few hours) after the second injection. All these 3 patients were positive for *E. coli* anti-asparaginase antibodies at the time of this second injection.

All other patients who achieved significant asparagine depletion duration were negative for *E. coli* anti-asparaginase antibodies at the time of the second injection.

Asparagine depletion <2 µM in cerebrospinal fluid

The number and timing of CSF asparagine measurements were highly variable. Some CSF samples were analysed when the patient had no more asparagine depletion in serum. Overall, 4 of 6 patients in the L-ASP group (66.7%) and 4 of 18 patients in the Graspa groups (22.2%) achieved asparagine depletion (<2 µM) in CSF during follow-up.

Table 2-8 Study GRASPALL 2005-01: Duration of asparagine depletion <2 µM after administration of the 1st GRASPA dose by age subgroup

Duration of depletion	Days	GRASPA 50 IU/kg (N=6)	GRASPA 100 IU/kg (N=6)	GRASPA 150 IU/kg (N=6)
Adults	**n**	**3**	**3**	**3**
Minimum duration: last point value <2 µM	Mean	4.93	6.30	12.03
	Min-Max	2.9-6.0	6.0-6.9	6.1-18.0
Maximum duration: first point value >2 µM	Mean	10.20	12.27	19.67
	Min-Max	5.8-12.9	11.9-12.9	13.0-27.0
Estimated duration	Mean	7.57	9.28	15.85
	Median	8.90	9.40	15.50
	Min-Max	4.35-9.45	9.0-9.45	9.55-22.50
Children	**n**	**3**	**3**	**3**
Minimum duration: last point value <2 µM	Mean	7.00	5.97	17.27
	Min-Max	3.0-12.0	5.9-6.0	5.8-40.0
Maximum duration: first point value >2 µM	Mean	12.40	11.60	25.33
	Min-Max	6.0-19.2	11.0-12.0	12.0-51.0
Estimated duration	Mean	9.70	8.78	21.28
	Median	9.00	8.85	9.45
	Min-Max	4.5-15.6	8.5-9.0	8.9-45.5

Source: CSR GRASPALL 2005-01 Appendix 21 (Appendix 8 – 21) in Module 5.3.5.1

Secondary pharmacology

The results showed an increase in aspartic acid and glutamic acid levels, and reduction in glutamine levels (Table 2-3, Table 2-4, and Table 2-5). These changes are consistent with the enzymatic activity of L-asparaginase, i.e. L-asparagine is cleaved with production of aspartic acid and L-glutamine is cleaved with production of glutamic acid. However, reduction in glutamine levels demonstrated high variability, irrespective of the dose and therefore irrespective of the duration in asparagine depletion. As glutaminase activity of the encapsulated asparaginase is expected to be the same as the free form, a possible explanation for such difference may be explained by a limiting capability of the RBC membrane to transport glutamine.

Table 2-3 Area under the Curve and variance for Glutamic acid

Patient #	L-ASPA	GRASPA 50	GRASPA 100	GRASPA 150
1	1412.50	485.50	1103.50	2466.50
2	915.00	733.00	455.50	685.00
3	2588.00	613.00	481.00	505.00
4	2484.50	511.00	607.00	541.50
5	542.50	481.50	484.50	1715.50
6	875.00	.	570.50	964.50
mean	1469.58	564.80	617.00	1146.33
S.D.	356.12	48.34	100.18	320.93
minimum	542.50	481.50	455.50	505.00
maximum	2588.00	733.00	1103.50	2466.50

Source : GRASPALL 2005-01 Clinical study Report - Table 10 in Module 5.3.5.1

Table 2-4 Area under the Curve and variance for Glutamine

Patient #	L-ASPA	GRASPA 50	GRASPA 100	GRASPA 150
1	2004.00	4848.50	4231.50	3590.50
2	3830.50	6280.00	4998.50	4246.00
3	1902.00	4642.50	3244.00	4948.50
4	1867.50	4074.50	4647.00	4932.00
5	3271.50	5085.50	3952.00	4370.00
6	3044.00	.	4025.50	4174.00
mean	2653.25	4986.20	4183.08	4376.83
S.D.	342.74	364.15	247.83	209.10
minimum	1867.50	4074.50	3244.00	3590.50
maximum	3830.50	6280.00	4998.50	4948.50

Source: GRASPALL 2005-01 Clinical study Report - Table 11 in Module 5.3.5.1

Table 2-5 Area under the Curve and variance for Aspartic acid

Patient #	L-ASPA	GRASPA 50	GRASPA 100	GRASPA 150
1	64.50	95.50	103.00	79.50
2	68.50	120.50	54.50	71.00
3	63.00	154.50	93.50	58.50
4	91.00	126.00	109.50	54.00
5	28.00	105.00	133.50	103.50
6	53.50	.	88.50	133.00
mean	61.42	120.30	97.08	83.25
S.D.	8.40	10.13	10.67	12.27
minimum	28.00	95.50	54.50	54.00
maximum	91.00	154.50	133.50	133.00

Source: GRASPALL 2005-01 Clinical study Report - Table 12 in Module 5.3.5.1

Relationship between plasma concentration and effect

The relationship between Graspa dose levels and response was not formally evaluated due to limited number of patients in the early Graspa clinical studies. There was an apparent increase in the pharmacodynamic effects (asparagine depletion and production of anti-L-asparaginase antibodies) with increasing doses.

3.3.3 – 3.3.4 Discussion and conclusion on clinical pharmacology

Pharmacokinetics

Overall, it appears the mean C_{max} and AUC_{inf} of encapsulated L-asparaginase increased with increasing doses from 50 to 150 IU/kg and mean $t_{½}$ of encapsulated asparaginase ranged from 15-18 days for the adults and children.

Pharmacokinetic parameters following the first infusion seem to be reasonably well characterised. However, pharmacokinetics for more than one infusion should be further characterised. The applicant mentions that ongoing and future studies will incorporate suitable sampling strategies to further characterise the PK following multiple infusions. These results should be submitted as post-commitment and deadlines should be specified.

Frequency of infusion mentioned in the SmPC (approximately every 2 to 3 weeks) has not been properly justified. Considering data for terminal $t_{1/2}$ (mean $t_{½}$ of encapsulated asparaginase ranged from 14-18 days), there is not complete washout of L-asparaginase between the first and second infusion, although the PK parameters following the first and second infusion in study GraspaLL 2005-01 seem to be similar for the first and second infusion of 150 IU/Kg. It should be kept in mind that this conclusion is based on data from 6 patients for the first infusion and 3 patients for second infusion. Figure 7 is from only 1 subject. Frequency of infusion mentioned in the SmPC should be further discussed and justified.

No relevant pharmacokinetic differences are apparently detected in the PK of L-asparaginase by age or gender. However, it should be kept in mind that the number of patients in some age categories is limited, especially for terminal $t_{1/2}$ analysis. For example, in AUC_{last} differences may be observed between 2-55 and 56-84 if high number of patients were included in categories 12-17 and 75-84. Therefore, no firm conclusion can be raised in relation with age due to limited number of patients in some age categories and results should be interpreted with caution. Caution about conclusions on no apparent pharmacokinetic differences in the PK of L-asparaginase by age should be reflected under section 5.2 in the SmPC.

Pharmacodynamics

The main discussion of the principal PD endpoint, asparagine depletion, occurs in the Efficacy section.

In the phase I PK/PD study (GraspaLL 2005-01), large variations in asparagine depletion were seen in all 4 study arms. However a dose-response effect was still clearly noted. There was a trend for longer duration in the patients administered L-asparaginase compared to patients administered Graspa. Overall, the data for various PD endpoints (duration of asparagine depletion, proportion of patients presenting with asparaginase depletion over time, incidence and duration of asparagine depletion in CSF) all show superior responses to treatment with L-asparaginase over Graspa. Consistent with this evidence of superior effect of L-asparaginase on asparagine are the data showing a much lower AUC for glutamine than those for any doses of Graspa. Indeed, there was little difference in AUC between the 3 doses of Graspa, which raises additional concern regarding the efficacy of Graspa, as some of the anti-leukaemic effect is postulated to be mediated through reduction in glutamine levels. Data from study GraspaLL 2005-01, together with PD data from the pivotal study, despite the small patient numbers, provide ample evidence of the superior PD responses achieved with L-asparaginase compared to Graspa.

Issues raised by the Applicant regarding shortcomings of the asparaginase assay have not been accepted. These are discussed in the Efficacy section.

For the pivotal Study, conversion to positive antibody status occurred in 8 (30.8%) patients treated with Graspa, in 7 (25.0%) patients treated with L-ASP and in 7 (26.9%) patients treated with Graspas. Median time to antibody positivity was not reached in both Graspa and L-ASP arms. The median time to antibody positivity was 0.6 months for Graspas. These results go against the claimed protection by encapsulation. Thus, there are uncertainties on to what extent encapsulation truly prevent from immunological reactions given the similar rate of antidrug antibodies formation and the higher incidence of SAE HS reactions in the Graspa treated arm. A discussion about possible reasons for the lack of difference in immunogenicity rates between the two main study arms in the pivotal trial was requested. This should have included a discussion of the potential for exposure of immune system

components to the asparaginase in Graspa – for instance through intravascular haemolysis, erythrocyte degradation in the reticuloendothelial system with associated release of free asparaginase in the spleen and liver and the presence of membrane bound asparaginase in erythrocytes. In relation with this issue, the applicant is aware that the assays used to assess antibody status are not optimal. Therefore, immunogenicity cannot be properly analysed. Consequently, one of the two goals of this clinical development (significantly less immunogenicity is expected with Graspa) is highly questioned at this time, although the rate of allergic reactions was so different for Graspa and L-ASP (in the pivotal trial, none of the patients in the Graspa arm had evidence of hypersensitivity reaction during induction compared to 46% of patients in the control arm, who experienced hypersensitivity reactions). Reliable data about immunogenicity should be provided. New analyses of the samples from the pivotal trial will be provided and timelines should be specified.

Potential effect on prolongation of QT has not been studied. However, considering the lack of prior safety concerns for QT prolongation with native L-asparaginase, no conduction of QT study is accepted.

3.3.4 Clinical efficacy

The evidence of the efficacy of Graspa in combination with chemotherapy for the treatment of children and adults with refractory or relapsed Ph- ALL, with or without evidence of prior hypersensitivity reactions, is provided by the results from the pivotal Phase II/III Study GraspaLL 2009-06. In addition to the pivotal study, supportive data are presented from Study GraspaLL/ GRAALL SA2-2008, a Phase IIa open label study conducted in elderly patients >55 years, presenting with newly diagnosed Ph- ALL. Additionally, supportive data are provided by Study GraspaLL 2005-01, a Phase I, open label study conducted to determine the recommended Phase II dose, safety, and pharmacokinetics (PK) of Graspa in combination with chemotherapy.

Table 2.7.3- 4 Summary of GRASPA studies with efficacy data

Study Number	Type of study/population	Population	Treatment	Status
GRASPALL 2005-01	Phase I/II, parallel-group study in adults and children aged ≥1 year, with ALL, in combination with multi-agent chemotherapy	Planned and treated: 24 GRASPA: 18 (9 children, 9 adults) Native L-ASP: 6 (3 children, 3 adults)	L-ASP or GRASPA 50 IU/kg, 100 IU/kg or 150 IU kg + COOPRALL	Completed (8 weeks treatment + 12 month follow-up)
GRASPALL/ GRAALL SA2-2008	Phase IIa, open-label, dose escalating study in ALL Ph- adults aged ≥ 55 years in combination with multi-agent chemotherapy	Planned: 9 to 30 (≥3 in each cohort); Treated: 30	GRASPA 50 IU/kg, 100 IU/kg or 150 IU kg[a] + EWALL	Completed (22 to 28 days treatment per induction + 24 month follow-up)
GRASPALL 2009-06	Phase II/III, parallel-group study in adults and children (1-55 years) with ALL in first relapse, in combination with multi-agent chemotherapy	Planned: 60 to 80 (54 children, 26 adults) Treated: 80: (57 children, 23 adults)	L-ASP or GRASPA 150 IU/kg + COOPRALL	Completed 1-year follow up; 2 year maintenance phase ongoing

Dose-response studies

Study GraspaLL 2005-01

Study Design and patient population

This was an open label European Phase I study, evaluating the safety and biological activity of Graspa in patients with relapsed ALL, aged between 1 and 55 years without known hypersensitivity to L-asparaginase. The primary objective of the study was evaluation of the dose of Graspa that induced a durable depletion of plasma asparagine levels.

Eligible patients were males or females aged < 55 years old, presented with relapsed or refractory Ph-ALL and ECOG PS score of 0,1 or 2. Patients who presented with 2nd relapse were excluded from the study.

Patients treated with COOPRALL chemotherapy protocol and randomly assigned before treatment initiation to either L-ASP 10,000 IU/m² every 3 days for 8 administrations during F1F2 blocks and 6 injections during first R2R1 block sequence or Graspa with one administration during F1F2 blocks and one injection during first R2R1 block. The study had 4-cohorts:

- A reference cohort of L-ASP administered intravenously or intramuscularly at the dose of 10,000 IU/m² every 3 days starting on Day 4 of F1 block (or Day 6 of VANDA block).

- Three cohorts of escalating doses of Graspa: 50, 100, and 150 IU/Kg, (2 administrations).

Study endpoints

The primary endpoint of the study was the duration of plasma asparagine depletion (<2 µM). Samples were analyzed at central laboratories.

The secondary efficacy endpoints of the study were: Pharmacokinetic assessments of free and encapsulated asparaginase, immunogenicity assessments, amino acid changes (serum L-aspartic acid, L-glutamine and glutamic acid), and CR rate. Safety assessments were carried throughout the study up to 4 months after the last study treatment administration (Graspa or L-ASP), and included clinical and laboratory parameters and reporting of AEs.

Statistical analysis

The Intent-To-Treat analysis population was all patients enrolled into the study, who received at least one dose of the study drug.

The primary endpoint was the duration of asparagine depletion $\leq$ 2 μM measured from the first administration of Graspa, to the last PK time. The sample size was calculated with the assumptions that target depletion duration increases with the dose. Therefore, 24 patients (12 adults and 12 children) were needed in order to compare the 3 Graspa groups (50, 100, and 150 IU/Kg).

An ANOVA for each age stratum was used, for a global comparison of the 3 groups (50, 100, and 150 IU/Kg), using an alpha of 5% and a power of 80%.

Primary efficacy analysis: Distribution of asparagine depletion was described between treatment groups. Since the interval between sample collection was rather variable during follow-up, a sensitivity analysis was performed on the primary endpoint using i) the first PK time with asparagine depletion value > 2 µM, and ii) a linear interpolation between the last PK time with asparagine depletion value < 2 µM and the following point.

Secondary efficacy analysis: For other pharmacokinetic and pharmacodynamic data, the first administration date of the investigational treatment was considered as the reference date for delay calculation.

The maximum mean concentration (C_{max}), the terminal half-life was calculated by the log-linear regression from the linear proportion of the logarithmic transformed concentration-time plot. Mean overall systemic exposure parameters (AUC) were also estimated.

Measurement of CSF asparagine level was planned each time a CSF sampling was performed during intrathecal injection. Sampling was at Day 2 of F1/Day 19 of F2 or Day 5 of VANDA for induction block and Day 2 of R1 and R1 consolidation block.

Study results

- Patient disposition

Of the 24 patients enrolled into this study, 18 patients received Graspa, with 6 patients in each dose level (50, 100, and 150 IU/Kg). Further 6 patients received L-ASP. Study drug was discontinued in 2 out of 6 patients at the dose level of 50 IU/Kg due to target asparagine depletion not reached. At the dose level of 100 IU/Kg, Graspa was discontinued in 2 patients due to related toxicity, and one patient due to pre-defined protocol procedure. At the dose level of 150 IU/Kg, Graspa was discontinued in one patient due to related toxicity, and one patient due to pre-defined protocol procedure, Table 2.7.3- 14.

Table 2.7.3- 14 Summary of patient disposition - GRASPALL 2005-01

	L-ASP N= 6	GRASPA 50 N= 6	GRASPA 100 N= 6	GRASPA 150 N= 6
Study drug discontinuation				
Target asparagine depletion not reached	0	2	0	0
Study drug related toxicity	4	0	2	1
Protocol non compliance	0	0	1	1
Chemotherapy protocol withdrawal				
BM Transplantation	3	1	1	0
Therapeutic failure	0	1	1	3
Survival				
Completed 1 year follow-up	4	5	5	3
Death	2	1	1	3

Source: Table in Section 6.1 of the CSR.

Protocol violations

The main protocol deviations were related to compliance with the study protocol. None of the deviations was considered major.

- Baseline demographics and disease characteristics

Of the 18 patients enrolled in the Graspa cohorts, 12 patients were males and 6 females, thus representing the general characteristics of the disease. There were 4 patients in the Graspa 150 IU/Kg who presented with very early relapse, compared to one patient each in the other Graspa cohorts, Table 2.7.3- 15.

Table 2.7.3- 15 Demographic characteristics - Study GRASPALL 2005-01

		L-ASP N=6	GRASPA 50 N=6	GRASPA 100 N=6	GRASPA 150 N=6
Gender	Male	3	3	6	3
	Female	3	3	0	3
Age (yrs, Mean/min- max)					
Children		7.9 (5.4-10.9)	9.1 (4.7-16.8)	10.5 (7.4-15.8)	10.3 (7.7-14.7)
Adults		31.2 (29.2-34.8)	34.0 (23-46.8)	22.3 (19.3-25.3)	34.3 (34.3-40.5)
Time of relapse (n)					
Very early [1]		0	1	1	4
Early [2]		1	2	2	0
Late [3]		5	3	3	2

- Efficacy results

PEP (see Pharmacodynamics)

Key secondary efficacy endpoints

-Amino acid changes (see Pharmacodynamics)

-CSF asparagine depletion (see Pharmacodynamics)

-CR Rate

Overall, after induction, 16 (66.7%) patients achieved CR, 4 patients (3 children and 1 adult) did not respond to the induction therapy, 3 patients achieved an incomplete remission after induction and continued the consolidation block, and 1 patient in the Graspa group with an initial diagnosis of isolated CNS, was considered by the investigator as non evaluable after induction treatment, continued the consolidation treatment. This patient experienced an isolated CNS relapse 2 months after the start of induction therapy.

Main clinical study

GraspaLL 2009-06 STUDY

Title: A multicentre, open, randomized, Phase II/III study, evaluating efficacy and safety of erythrocytes encapsulating L-asparaginase (Graspa) versus reference L-asparaginase treatment in combination with standard polychemotherapy in patients with first recurrence of Philadelphia chromosome negative acute lymphoblastic leukaemia.

Administrative information

Active sites:

France - 25 active sites in France/ 2 active sites in Belgium

Objectives

To determine the efficacy and safety of ERY001 at a dose equivalent to 150 IU/Kg of L-asparaginase, when combined with standard multi-agent chemotherapy for the treatment of patients with first recurrence of Philadelphia chromosome negative acute lymphoblastic leukaemia (Ph- ALL), with or without known hypersensitivity to L-asparaginase.

Methods

Study design

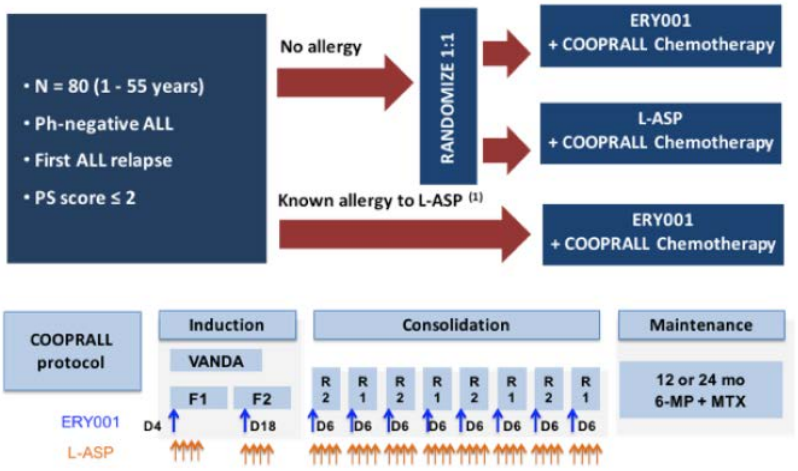
The study was designed as an adaptive Phase II/III trial, in which the Phase II outcome informed decision for the Phase III stage, with regards to patient population and overall study design.

All patients were screened for history of prior hypersensitivity reactions to native E Coli L-asparaginase (NCI-CTCAE Grade 1 to 3 toxicity; Grade 4 hypersensitivity reaction was an exclusion criterion for the study).

The study has two controlled arms and one single stratum:

- The non-allergic patients (history of allergic reaction Grade <2) were randomized to receive either ERY001 or L-ASP
- The allergic patients (history of allergic reaction Grade ≥2) were treated with ERY001 (single arm, ERY001-s)

Figure 5-1 Trial diagram



All patients received the COOPRALL schedule as backbone chemotherapy. It consisted of successive blocks of multi-agent chemotherapy. Treatment was stratified into 4 groups using the "risk scoring according International Berlin Frankfurt Munich (IBFM)-study group": S1, S2, S3, and S4.

S1-S2 (moderate risk) groups received F1 chemotherapy block on Day 1, and F2 chemotherapy block on Day 15.

S3-S4 (high risk) groups received VANDA chemotherapy block

Following F1-F2/VANDA chemotherapy blocks, all groups received R2 and R1 after regeneration of BM aplasia, followed by maintenance (12 to 24 months), with oral low dose methotrexate and 6-Mercaptopurine.Study treatment phase with investigational product averaged between 2.5 to 6 months depending on relapse severity and treatment response.

The study follow-up lasted 12 months.

A pre-phase of 2 to 5 days (up to maximum 10 days) of dexamethasone (6 mg/m²/d) treatment (pre-phase) was generally given to prepare patients to intensive chemotherapy afterwards. Central Nervous System (CNS) prophylaxis was also given the first day at the beginning of pre-phase.

ERY001 was administered at the dose equivalent to 150 IU/Kg of L-asparaginase on Day 4 and 18 of F1-F2 blocks, or on Day 6 of VANDA block, then on Day 6 of R2/R1 block in all patients.

L-ASP was administered intravenously or intramuscularly at the dose of 10,000 IU/m² every 3 days starting on Day 4 of F1 block (or Day 6 of VANDA block) in non-allergic patients. The dosing schedule followed the currently approved label for the native E. Coli L-asparaginase. Patients were followed regularly during induction and consolidation, then during the follow-up visits at approximately 6 months (M6), M12, M24 and M36. Trial assessment lasted 36 months for each patient. Follow-up beyond 12 months is not available at this time.

Safety assessments were carried throughout the study up to 4 months after the last study treatment administration (ERY001 or L-ASP).

Day 1 (D1) refers to the start of the induction chemotherapy.

Schedule of assessments

F1/F2 induction

Table 5-1 Calendar of study assessments

INDUCTION	**selection**	**F1**														**F2**													
Week		**1**							**2**							**3**							**4**							
Day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Visit		V1 incl			V2			V3								V4			V5			V6							
Consent		X																											
Demographics		X																											
Inclusion/exclusion criteria	X	X																											
Medical history		X																											
ALL history		X																											
Randomization		X																											
Clinical assessment (a)		X						X								X						X							
Chemotherapy (b)	Prephase	X	X	X	X	X	X	X								X	X	X	X	X										
Native L-asparaginase (L-ASP)(c)					X			X			X			X					X			X			X			X	
ERY001 (c)			P		X												P		X										
MRD / Myelogram	X																												
Thrombolytic balance		X																											
Biology (d)		X			X			X			X			X		X			X			X			X			X	
Plasma amino acids (e)					X	X		X			X			X					X	X		X			X			X	
Asparaginase (e)					X	X		X			X			X					X	X		X			X			X	
Anti-L-asp antibodies (e)					X									X					X									X	

Occurrence of AE/SAE and changes in concomitant treatment were to be recorded throughout the study

(a): complete or partial (physical examination),

(b): for daily treatment details, refer to COOPRALL guidelines, section 10 of CSP

(c): patient to be randomized to receive either Native L-asparaginase (L-ASP) (10 000 IU/m²) or ERY001 (150 IU/Kg)

(d): hematology (CBC); chemistry; coagulation parameters (refer to section 8.5.3 of CSP)

(e): sample had to be taken prior to administration of Native L-asparaginase (L-ASP)(any form)

P: prescription

VANDA Induction

INDUCTION	selection	VANDA																										
Week		1							2							3							4						
Day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Visit		V1 incl					V2			V3						V4			V5			V6							
Consent	X																												
Demographics		X																										
Inclusion/exclusion criteria	X	X																										
Medical history		X																										
ALL history		X																										
Randomization		X																										
Clinical assessment (a)		X								X						X						X						
Chemotherapy (b)	prephase	X	X	X	X	X	X																						
Native L-asparaginase (c)							X			X			X			X													
ERY001 (c)					P		X																						
MRD/Myelogram	X																												
Thrombolytic balance		X																											
Biology (d)		X					X			X			X			X						X					X	
amino acids (plasma) (e)							X	X		X			X			X						X					X	
Asparaginase (e)							X	X		X			X			X						X					X	
anti-L-asp antibodies (e)							X									X											X	

Occurrence of AE/SAE and changes in concomitant treatment were to be recorded throughout the study

(a): complete or partial (physical examination),

(b): for daily treatment details, refer to COOPRALL guidelines, section 10 of CSP

(c): patient to be randomized to receive either Native L-asparaginase (10 000 IU/m²) or ERY001 (150 IU/Kg)

(d): hematology (CBC); chemistry; coagulation parameters (refer to section 8.5.3 of CSP)

(e): sample had to be taken prior to administration of L-asparaginase (any form)

P: prescription

Rationale - Study design and Interim analysis

This study was planned as an adaptive Phase II/III study, consisting of the following three parts:

• A Phase II study, which was initially planned with a sample size of 30 patients. It was expected that among these 30 patients there were 20 non-allergic and 10 allergic patients.

• An interim analysis to inform decision for patient selection and ultimate sample size for the confirmation Phase III trial. The statistical considerations were based on a Bayesian analysis combining efficacy and toxicity of ERY001.

• A confirmatory Phase III study. The final analysis was to be based on all patients enrolled in the entire study.

The planned interim analysis took place on 05th July 2011, following the enrolment of the planned 30 patients. Due to too small number of patients, the DSMB concluded that this interim analysis was not informative and recommended to continue the trial without modification but to add a second interim analysis after a total of 60 patients had been enrolled.

The second interim analysis took place on 14th March 2013, after 65 patients had been treated. The DSMB concluded that the study should be continued to enrol up to 80 patients. No modifications to patient population or primary endpoints were recommended.

The main rationale for the adaptive design of this study was to ascertain the assumptions based on previous trials regarding the rate of allergies and mean depletion duration with ERY001. Consequently, the study was planned as such, so that study sample size adjustment could be made if necessary, thus avoiding the risk of being underpowered, and increasing the ability to achieve its goal, in an orphan disease.

All statistical details are presented in the statistical analysis plan. The DSMB had the potential to stop the study for either efficacy or for futility.

It is recognized that the DSMB as constituted was acting more as an Expert Panel advising the sponsor on the conduct of the study. The potential for bias, in terms of the control of type I error was assessed in the final analysis through Fishers combination test for the co-primary endpoints. The potential for operational bias was evaluated by looking at data for both the primary efficacy and the primary safety

endpoints separately within the 3 parts of the trial; patients recruited before the first meeting of the DSMB, those recruited between the first and the second DSMB meetings and finally those recruited after the second DSMB meetings.

Chemotherapy treatment protocols

Table 5-2 Induction treatment schedule

Block	F1														F2													
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Dexamethasone,10 mg/m²/bid	X	X	X	X	X										X	X	X	X	X									
Vincristine, IVD (max 2 mg) 1.5 mg/m²/d	X					X									X													
Methotrexate, IV: 1 or g/m² /24h with CNS involvement	X																											
Cytarabine IV in 3h , 3g/m²/bid															X	X												
Intrathecal CNS preventive therapy: Methotrexate, Cytarabine Prednisone		X					**												X									
Native L-ASP,10,000 UI/m²				X			X			X			X					X			X			X			X	
ERY001 150 IU/Kg				X														X										
Plasma amino- acids, Asparaginase				X	X		X			X			X					X	X		X			X			X	
Anti-L-asp antibodies				X									X					X									X	

Block	VANDA																											
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Dexamethasone,10 mg/m²/bid	X	X	X	X	X	½																						
Cytarabine IV in 3h , 2g/m²/bid	X	X																										
Mitoxantrone IV in 2h, 8mg/m²/d			X	X																								
Etoposide IV in 1h, 150mg/m²/d			X	X	X																							
Intrathecal CNS preventive therapy : Methotrexate, Cytarabine Prednisone					X																							
Native L-ASP,10,000 IU/m²						X			X			X			X													
ERY001 150 IU/Kg						X																						
Plasma amino- acids, Asparaginase						X	X		X			X			X						X						X	
Anti-L-asp antibodies						X									X												X	

Patients

Inclusion Criteria

- 1 to 55 years old
- 1st ALL relapse, which could be either isolated bone marrow relapse, or combined (medullary and extra-medullary) relapse, or extra-medullary isolated relapse; or lymphoblastic lymphoma (except Burkitt's lymphoma),

  OR

  Failure to ALL first line treatment (no complete remission obtained).
- previously treated with free or PEGylated E.coli L-asparaginase
- Performance Status ≤2 (ECOG score),

Exclusion Criteria

- ALL t(9;22) and/or BCR-ABL positive (Philadelphia chromosome positive)
- Patient with 2nd relapse and over
- Women of childbearing potential without effective contraception as well as pregnant or breast-feeding women

- Patient unable to receive treatments used in global chemotherapy protocols, due to general or visceral conditions such as:
    - Severe cardiac impairment (NYHA grade 3 or 4 cardiomyopathy)
    - Serum creatinine 2 x upper limit of normal (ULN) unless related to ALL
    - ALT or AST 5 x ULN unless related to ALL
    - History of pancreatitis
- Known Grade 4 allergic reaction to E.coli L-asparaginase (according NCI-CTCAE, Version 3.0)
- History of Grade 3 transfusion reactions
- Presence of specific anti-erythrocyte antibodies preventing from getting a compatible erythrocyte concentrate
- Patient under concomitant treatment likely to cause haemolysis
- Patient under phenytoin treatment

Endpoints

Primary efficacy endpoint

The revised efficacy co-primary endpoint was defined as:

- The duration in days of asparaginase activity >100 IU/L. Initially, asparagine activity was determined in the plasma in the L-ASP arm, and in whole blood for the ERY001 arm. Following CHMP advice on 24th November 2014, reporting of asparaginase activity levels were provided based on whole blood measurement (total asparaginase levels) in all treatment arms.
- incidence of hypersensitivity reactions

It should be noted that the revised endpoint was implemented following the completion of enrolment. Therefore, the mean duration of asparagine depletion was performed as the original primary endpoint, and was defined as plasma asparagine concentration ≤ 2 µM. The results of asparagine depletion levels were provided to the investigator, to inform decision regarding further management of the patient.

Secondary efficacy endpoints

- Plasma concentrations of amino acids (aspartate, glutamine, glutamate)
- Specific anti-L-asparaginase antibody level
- Complete Remission (CR) rate
- Percentage of CR patients at the end of F2 or VANDA block.

  Disease evaluation was performed at baseline, end of F2/VANDA block (Day 28), R2 block (Day 1), every 6 weeks at the end of R1 blocks, then at 6-, 12-, 24-months visits, and at end of study (36-months).

  A CR is defined as - No physical evidence of leukaemia and a normal complete blood cell count (CBC), Cytologic remission (Normally regenerating bone marrow, with < 5% leukemic blasts) and the absence of detectable CNS or extramedullary disease, evaluated with physical examination and Cerebrospinal fluid (CSF) finding. Only information regarding CBC and blast % were collected.

The absence of detectable CNS or extra-medullary disease was as judged by the investigator, and therefore, the detailed information regarding was not collected in the CRF pages.

- Minimal residual disease at the end of F2 or VANDA block (MRD)

  MRD is the presence of leukemic cells below the threshold of detection. For the purpose of this study, the value of 10^{-3} was taken as the threshold to indicate a negative ($<10^{-3}$) or positive ($>10^{-3}$) MRD.

  Analyses were performed at baseline, at the end of Induction phase (F2 block or VANDA ending), then every 6 weeks (at the end of R1 blocks, up to 3 samples). Analyses were performed at the local laboratories according local institutional practice, which could have utilized different assay techniques. However, the actual MRD test results were also directly provided by the local laboratory, and therefore were not collected in the CRF pages.

- Overall Survival (OS)

  Survival status was assessed by the investigator 6-, 12-, 24- and 36 months after the patient inclusion.

- Event Free Survival (EFS)

  The time from randomization until the first documented sign of disease relapse or death due to any cause. Also, patients with missing CR assessment were considered as a failure.

- Disease Free Survival (DFS)

  The time from randomization until the first documented sign of disease relapse. DFS was censored for allograft procedures.

Safety Variables

In addition to incidence of hypersensitivity reactions as a co-primary endpoint in the study, regular safety assessments included recording of AEs and SAEs, laboratory measurements and vital signs.

- Adverse Events (AE)
- Serious Adverse Events (SAE)
- Suspected Unexpected Serious Adverse Reactions (SUSAR)
- Clinical laboratory evaluations

Statistical methods

The CRO PM was in charge of all the statistical analyses.

The analysis of the primary criterion was performed with the R® software Version 2.15. All the other criteria were analysed with the SAS® software Version 9.2.

The final Statistical Analysis Plan (SAP) was issued on 22nd September 2014.

There were two Addenda to the SAP and these were issued on 24th October 2014 and 27th March 2015.

Co-Primary endpoints

The primary efficacy endpoint was the mean duration of asparaginase activity >100 IU/L in days, as measured in whole blood, throughout F1-F2 (or VANDA) blocks (induction period) and the pre-specified primary safety endpoint was the binary endpoint, presence/absence of allergic reaction (related to treatment, independent of grade), throughout F1-F2 (or VANDA) blocks. The statistical significance on both of these endpoints, at the one-sided 2.5% level of significance when considering ERY001 and L-ASP in the non-allergic subgroup, was required for a positive study.

The objective of the efficacy comparison was to demonstrate the non-inferiority of ERY001 treatment compared to L-ASP. Non-inferiority has been defined in terms of the ratio of the mean duration of asparaginase activity >100 IU/L values (ERY001/ L-ASP) being $\geq$ 0.80. This has been evaluated by constructing the 95% confidence interval for this ratio and declaring non-inferiority (at the one-sided 2.5% level of significance) if the interval is completely above 0.80. Should non-inferiority be demonstrated, then the 95% confidence intervals were to be inspected for a conclusion of superiority. Should the intervals lie completely above 0, then there is evidence of superiority at the one-sided 2.5% level of significance. A p-value for superiority was to be obtained using ANOVA with disease severity (and treatment) as factors in the model.

In case of missing value(s) for asparaginase activity, in absence of any ERY001 or L-ASP injection, the missing value(s) will be considered >100 IU/L if both the previous and the next available asparaginase activity are >100 IU/L, and $\leq$100 IU/L if either the previous or the next available asparaginase activity is $\leq$100 IU/L. In case of missing data that makes the calculation of the duration of asparaginase activity impossible for any injection, the duration will be set to 0 for that injection. Asparaginase levels were not measured following switch of treatment and these rules imply that in such cases the level is set to 0 following the switch.

The analysis of the primary safety endpoint was based on the Cochran-Mantel-Haenszel (CMH) test stratified by disease severity and the magnitude of the treatment effect for ERY001 treatment is presented through the 95% confidence interval for the difference (ERY001 minus L-ASP) in the proportions of patients suffering allergic reactions.

Exploratory Phase

The mean depletion duration is modelled by means of a parametric survival model, namely the Weibull model, which allows to model monotone hazards, i.e. increasing, constant and decreasing hazards. The allergy occurrence is modelled by means of a logistic model.

Non-allergic patients

The two marginal models are combined by applying the total probability theorem:

$$F_{E,T}(t, r) = Pr(E \leq t \mid T = r)\, Pr(T = r) = F_E(t \mid T = r)\, p\,; r = 0, 1\,; t \geq 0,$$

where E is the mean depletion duration, FE is the Weibull distribution function, p is the allergy rate. This then allows computing the probability that a Graspa® patient depletes longer and has fewer allergies than a reference L-asparaginase patient has.

Allergic patients

The model is similar to the model for non-allergic patients with the difference that there is no control group.

Sensitivity analyses looked at the primary efficacy endpoint were based on different thresholds for the definition of asparaginase activity, namely 75 and 400 IU/L in order to evaluate the robustness of the results to the choice of asparaginase cut-off set at 100 IU/L for the primary endpoint. An evaluation of

the total duration of asparaginase activity >100 IU/L was based on the construction of Kaplan-Meier curves with a formal non-inferiority comparison for ERY001 compared to L-ASP. The non-inferiority margin for the hazard ratio (HR) calculated using the proportional hazards model used a 'working guide' value of 1.25 with non-inferiority being declared if the 95% confidence interval for the HR (ERY001/ L-ASP) was <1.25.

For the analysis of the primary safety endpoint, two sensitivity analyses have been undertaken. The first analysis took account of all allergic events and not just those considered as related to treatment. Data on the primary safety endpoint, presence/absence of allergy, was collected throughout the 28 day treatment period, irrespective of treatment discontinuation and treatment switching, and a further sensitivity analysis counts all events during the 28 day period. This provides a conservative analysis for ERY001; in cases where a patient randomized to ERY001 switched to an alternative L-asp treatment, any allergies suffered following the switch were counted and assigned to ERY001 treatment.

It is recognized that this study was originally planned based on an adaptive design. The main methods of analysis and interpretation have ignored this structure. For completeness and transparency however the data for the primary efficacy and safety endpoints have been analysed based on methods that respect the adaptive nature of the design using Fishers combination test. Summary statistics for each endpoint are provided for each of the three parts of the trial to judge consistency in order to investigate the presence of operational bias caused by any lack of confidentiality of interim results.

Secondary endpoints

The duration of asparagine depletion ($\leq 2\ \mu M$) has been analysed using the same methods as for the primary analysis of the duration of asparaginase activity >100 IU/L.

Kaplan-Meier curves have been produced for overall survival, event-free survival and time to relapse together with hazard ratios and associated 95% confidence intervals. P-values comparing ERY001 and L-asp have also been calculated based on the log rank test.

The remaining secondary endpoints were evaluated using summary statistics and 95% confidence intervals for treatment differences where appropriate. P-values for treatment comparisons have been calculated in some cases but these will only constitute exploratory findings supportive of the findings from the co-primary endpoints.

All analyses have been undertaken on the Treated Population (TP), which comprised all patients enrolled into the study that have received at least one injection of investigational or reference medication.

Handling Of Dropouts Or Missing Data

Patients who did not receive the treatment, who were not evaluable for primary endpoint or withdrew their consent prior to receive study treatment (either ERY001 or L-ASP) were replaced.

For the primary endpoint of the mean duration of asparaginase activity, in case of missing value(s), if both the previous and the next available values were >100 IU/L, then the missing value was considered >100 IU/L. If both the previous and the next available values were ≤100 IU/L, then the missing value was considered ≤100 IU/L.

Similar approach was also used for handling missing values for the duration of asparagine depletion.

Missing values for other endpoints were not replaced.

Changes In The Conduct Of The Study Or Planned Analyses

Protocol Amendments

Three protocol amendments were issued for this study.

Protocol Amendment 3 was the most significant. It was a country-specific amendment, which was applied in France and Belgium. It modified the co-primary endpoints from the duration of asparagine depletion to the duration of asparaginase activity, based on the CHMP advice on 26th June 2014. In addition, based on Paediatric Committee (PDCO) request, this amendment included the analysis of patients with asparagine depletion and who do not have an allergic reaction.

Changes In The Statistical Analyses

The first Statistical Analysis Plan (SAP) was the version 03 dated 20th June 2011, it was produced after the first data review meeting which occurred on 12th May 2011.

A meeting between ERYTECH and the CHMP Scientific Advice Working Party took place on 4th June 2014. Upon receipt of the final CHMP Protocol Assistance letter dated 26th June 2014, a protocol amendment was finalized and the previous version of the SAP was modified to reflect the protocol amendment 3 (above). The final SAP was issued on 22nd September 2014. There were in addition two addenda issued on 24th October 2014 and 27th March 2015.

Interim analyses

The Interim Analysis was initially planned at the end of the recruitment in the Phase II part of the study to allow transition to Phase III.

This took place on 5th July 2011, after 31 patients have been treated. The database was locked on 21st June 2011. Due to the small sample size, the DSMB concluded that this interim analysis was not informative and recommended to continue the trial without modification and also asked for additional review after a total of 60 patients were enrolled. The recommendation was endorsed by ERYTECH Pharma.

Following this first interim analysis, the DSMB also recommended including the standard frequentist approach for primary criteria analysis.

The additional review after 65 patients enrolled took place on 14th March 2013. The database was locked on 11th February 2013. The DSMB recommended that the study continue until its end and that up to 80 patients were to be enrolled. Thus, the final analysis was performed on 80 patients.

Results

Patient disposition

Figure 6-1 Patient disposition

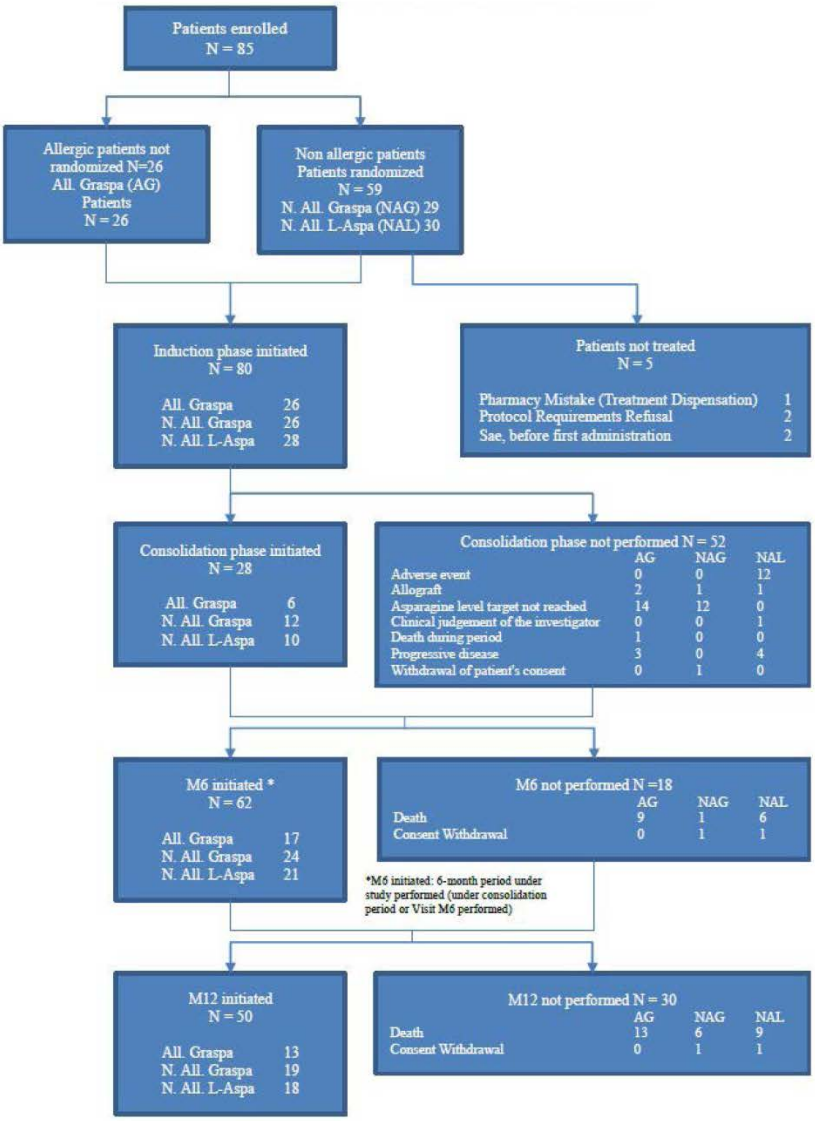


Table 6-1 Summary of patients disposition – Treated population

		ERY001 N (%)	L-ASP N (%)	ERY001-s N (%)	Total N (%)
Enrolled		29 (100)	30 (100)	26 (100)	85 (100)
Treatment not received [1]		3 (10.3)	2 (6.7)	0	5 (5.9)
Received Study Treatment		26 (89.7)	28 (93.3)	26 (100)	80 (94.1)
Safety analysis set		26 (100)	28 (100)	26 (100)	80 (100)
Treated analysis set		26 (100)	28 (100)	26 (100)	80 (100)
PP analysis set		26 (100)	27 (96.4)	26 (100)	79 (98.8)
Analysis subsets					
1- Risk groups	F1/F2 block	16 (61.5)	15 (53.6)	12 (46.2)	43 (53.8)
	VANDA block	10 (38.5)	13 (46.4)	14 (53.8)	37 (46.3)
2- Age groups	Children n (%)	21 (80.8)	21 (75)	15 (57.7)	57 (71.3)
	[1; 11[	19 (73.1)	13 (46.4)	10 (38.5)	42 (52.5)
	[11; 18[	2 (7.7)	8 (28.6)	5 (19.2)	15 (18.8)
	Adults [18-55]	5 (19.2)	7 (25)	11 (42.3)	23 (28.8)
Induction treatment completed		17 (65.4)	13 (46.4)	10 (38.5)	40 (50)
Treatment discontinuation with study drug		25 (96.1)	26 (92.9)	26 (100)	77 (96.3)
During induction		**14**	**18**	**20**	**52**
	Asparagine depletion target level not reached	12 (85.7)	0	14 (70.0)	26 (50.0)
	Progressive disease	0	4 (22.2)	3 (15.0)	7 (13.5)
	Adverse event	0	12 (46.2)	0	12 (23.1)
	Allograft	1 (7.1)	1 (5.6)	2 (10.0)	4 (7.7)
	Death	0	0	1 (5)	1 (1.9)
	Others [2]	1 (7.1)	1 (5.6)	0	2 (3.8)
During consolidation		**11**	**7**	**6**	**24**
	Asparagine depletion target level not reached	4 (36.4)	0	1 (16.7)	5 (20.8)
	Progressive disease	0	1 (14.3)	1 (16.7)	2 (8.3)
	Adverse event	1 (9.1)	3 (42.9)	1 (16.7)	5 (20.8)
	Allograft	6 (54.5)	2 (28.6)	3 (50.0)	11 (45.8)
	Other	0	1 (14.3)	0	1 (4.2)
12 month follow-up completed		19 (73.1)	18 (64.3)	13 (50)	50 (62.5)
Death		6 (23.1)	9 (32.1)	13 (50)	28 (35.0)
Consent withdrawal		1 (3.8)	1 (3.6)	0	2 (2.6)
COOPRALL compliance [3]					
Yes		18 (69.2)	14 (50)	13 (50)	45 (56.3)
No		8 (30.8)	14 (50)	13 (50)	35 (43.8)
Switch to other L-ASP [4]					
During induction		3 (11.5)	8 (28.6)	6 (23.1)	17 (21.3)
During consolidation		10 (38.5)	5 (17.9)	8 (30.8)	23 (28.8)
No Switch		12 (46.2)	14 (50.0)	12 (46.2)	38 (47.5)
Missing values [5]		1 (3.8)	1 (3.6)	0	2 (2.5)

N corresponds to the number of patients in treatment groups
[1] Pharmacy error (n=1), Protocol requirements refusal by investigator after randomization (n=2), and SAE before first administration (n=2);
[2] Other causes for treatment discontinuation included investigators decision, and consent withdrawal;
[3] COOPRALL compliance was collected until patient withdrew from the allocated asparaginase treatment, data regarding COOPRALL received after switch are not available;
[4] Patients 106-02 and 201-01 switched from L-ASP to ERY001;
[5] Patients 120-01 and 125-10 withdrew their consent during the consolidation period
Source: Table 14.1.1. , Table 14.1.2
Additionally, disposition of the patients by age (children/ adults) and by antibodies to L-ASP is provided in Tables 14.1.2 & Table 14.1.3.

Treatment discontinuation with study drug grouped all withdrawals that occurred after induction but prior to consolidation with those which occurred before end of induction. In order to provide a clearer account on the performance of both Graspa and L-ASP, treatment discontinuation has been accounted for the three treatment periods: during induction; between end of induction but prior to consolidation; and those which occurred during consolidation. The revised frequencies in relation to these entries are given in Table 8. In the randomised groups, a total of 18/54 (33.3%) patients discontinued study treatment during induction; 4/26 (15.4%) in the Graspa group and 14/28 (50.0%) in the L-ASP group. Of the 4 discontinuations in the Graspa group, 3 were for 'target depletion of asparagine level not reached' and one was 'consent withdrawn' during the consolidation phase. In the L-ASP group 12 patients discontinued due to 'adverse event', one due to 'progressive disease' and one due to 'investigator decision.

Table 8 Summary of Patients Disposition – Treated Analysis Set

	GRASPA N = 26(%)	L-ASP N= 28 (%)	GRASPA-s N =26 (%)
Induction treatment completed	22 (84.6)	14 (50.0)	20 (76.9)
Treatment discontinuation with study drug during induction	4 (15.4)	14 (50.0)	6 (23.1)
Reasons for discontinuing during induction			
Progressive disease	0 (0.0)	1 (7.1)	0 (0.0)
Adverse event	0 (0.0)	12 (85.7)	0 (0.0)
Consent withdrawal	1 (25.0)	0 (0.0)	0 (0.0)
Investigator decision	0 (0.0)	1 (7.1)	0 (0.0)
Target depletion of asparagine level not reached	3 (75.0)	0 (0.0)	6 (100.0)
Patients who discontinued treatment prior to consolidation (post-induction)	10 (40.0)	4 (16.0)	14 (53.8)
Patients who discontinued treatment during consolidation	11 (44.0)	7 (28.0)	6 (23.1)
Patients starting consolidation treatment	12 (46.2)	10 (35.7)	6 (23.1)
Switch to other L-ASP			
During induction	3 (11.5)	8 (28.6)	6 (23.1)
During consolidation	10 (38.5)	5 (17.9)	8 (30.8)
No Switch	12 (46.2)	14 (50.0)	12 (46.2)
Missing values	1 (3.8)	1 (3.6)	0

Source: Appendix 14 – version 1.1 – 15-Jun-16 - Table 14.1.1

The switching rate and the time of switching are considered more relevant that drug discontinuation for the assessment of PD and efficacy parameters. Both were similar throughout the study in the randomized treatment arms: 13/28 (46%) in the L-ASP arm and 14/26 (54%) in the Graspa arm.

- 4 patients in the Graspa arm switched after F1 while 5 patients in the L-ASP arm switched during or after F1;
- 8 patients in the Graspa arm switched after induction while 5 patients switched during or after F2/VANDA;
- 2 patients in the Graspa arm vs 3 patients in the L-ASP arm switched during consolidation.

Importantly, the proportion of patients starting consolidation treatment was slightly higher in the randomised Graspa arm (12/26; 46%) than in the L-ASP arm (10/28; 36%), but much lower in allergic patients (6/26; 23%).

Baseline demographics and disease characteristics

Table 7-2 Baseline demographic characteristics - Treated analysis set

		ERY001 N =26 (%)	L-ASP N =28 (%)	ERY001-s N =26 (%)	Total N =80 (%)
Gender	Male	16 (61.5)	18 (64.3)	18 (69.2)	52 (65)
	Female	10 (38.5)	10 (35.7)	8 (30.8)	28 (35)
Age (years)	Mean (SD)	12.3 (10.5)	16.3 (13.2)	19.2 (15.9)	15.9 (13.5)
	Median (range)	8.7 (2.7- 42.4)	12.5 (1.9- 55.9)	12.8 (2.1- 56)	10.6 (1.9 - 56)
Age group (years)	**[1; 11[**	**19 (73.1)**	**13 (46.4)**	**10 (38.5)**	**42 (52.5)**
	Mean (SD)	7.2 (2.4)	6.9 (2.7)	6.4 (2.9)	7.0 (2.7)
	Median (range)	6.6 (2.7-10.8)	7.3 (1.9-10.4)	6.6 (2.1-10.6)	6.9 (1.9- 10.8)
	[11; 18[	**2 (7.7)**	**8 (28.6)**	**5 (19.2)**	**15 (18.8)**
	Mean (SD)	14.3 (3.5)	15.2 (1.9)	12.5 (1.1)	14.2 (2.2)
	Median (range)	14.3 (11.9-16.8)	15.7 (11.8-17.4)	12.1 (11.1- 13.6)	13.6 (11.1-17.4)
	[18; 55]	**5 (19.2)**	**7 (25.0)**	**11 (42.3)**	**23 (28.8)**
	Mean (SD)	31.0 (9.5)	34.6 (13.6)	33.9 (14.0)	33.5 (12.6)
	Median (range)	29.2 (20.8-42.4)	30.6 (18.8-55.9)	30.4 (19.8-56.0)	30.4 (18.8-56.0)
ECOG PS [1]	0	20 (76.9)	18 (64.3)	14 (53.8)	52 (65.0)
	1	4 (15.4)	8 (28.6)	9 (34.6)	21 (26.3)
	≥2	2 (7.7)	2 (7.1)	3 (11.5)	7 (8.8)
Weight (kg)	Age group				
	[1; 11[	**19**	**13**	**10**	**42**
	Mean (SD)	29.0 (11.6)	24.0 (6.3)	24.6 (8.9)	26.4 (9.7)
	Median (range)	25.8 (14- 52)	25.2 (10.3- 34)	25.3 (11.8- 39.2)	25.5 (10.3- 52)
	[11; 18[	**2**	**8**	**5**	**15**
	Mean (SD)	54.5 (3.3)	63.9 (7.8)	40.7 (8.3)	54.9 (13.0)
	Median (range)	54.5 (52.2- 56.8)	62.5 (52.7- 78)	42.9 (30- 51.4)	56.8 (30- 78)
	[18; 55]	**5**	**7**	**11**	**23**
	Mean (SD)	82.4 (14.1)	66.6 (13.2)	70.3 (16.7)	71.8 (15.6)
	Median (range)	80 (65- 104)	60 (55- 89)	66 (47- 109)	66 (47- 109)

N corresponds to the number of patients in treatment groups
Decimal digits were rounded to one decimal point for mean, median and SD
[1] Performance status score was based on WHO scoring system in the protocol. However, the data outputs referred to ECOG performance score. Both WHO and ECOG scoring system are identical.
Source: Table 14.1.5, Table 14.1.6, and Table 14.1.15, Listing Appendix 16.2.4
Additionally, Tables of ECOG PS by Children/Adults, by age group and by Antibody status at baseline are displayed in Tables 14.1.16, 14.1.17 and 14.1.18

Table 7-3 Baseline ALL history –Treated analysis set

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Time since primary diagnosis (mo)	Mean (SD)	32.1 (17.3)	36.4 (23.6)	28.0 (21.9)
	Median	33.7	32.0	23.4
	Range	3.7- 67.7	3.9- 94.1	3.8- 71.4
Immunophenotype	B cell	21 (80.8)	28 (100)	21 (80.8)
	T cell	5 (19.2)	0	5 (19.2)
Abnormal cytogenetics				
	Yes, n	9 (34.6)	10 (35.7)	8 (30.8)
	No, n	17 (65.4)	18 (64.3)	18 (69.2)
Translocation	**Yes, n**	**12 (46.2)**	**9 (32.1)**	**10 (38.5)**
	t(4;11)	1 (8.3)	2 (22.2)	5 (50.0)
	t(12;21)	3 (25.0)	1 (11.1)	0
	Other	8 (66.7)	6 (66.7)	5 (50.0)
	No, n	**14 (53.8)**	**19 (67.9)**	**16 (61.5)**
Allergic reactions to prior L-Asparaginase	**Yes, n**	**2 (7.7)**	1 (3.6)	**26 (100)**
	Grade 1	2 (100)	1 (100)	0
	Grade 2	0	0	20 (76.9)
	Grade 3	0	0	6 (23.1)
	No, n	**24 (92.3)**	**27 (96.4)**	**0**
Antibody Status [1]	**Negative, n**	20 (76.9)	21 (75.0)	11 (42.3)
	Positive, n	6 (23.1)	7 (25.0)	15 (57.7)

N corresponds to the number of patients in treatment groups
Decimal digits were rounded to one decimal point for mean, median and SD.
[1] n=5 patients had results for antibody levels as 'doubtful', which were re-assigned to either negative or positive by arbitration.
Source: Table 14.1.9, Listing Appendix 16.2.4
Additionally, characteristics ALL by age group, antibody status are displayed in Tables 14.1.10 to 14.1.12.

Table 7-4 Type of relapse and risk score –Treated analysis set

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Relapse time	On therapy	9 (34.6)	10 (35.7)	12 (46.2)
	Off therapy	17 (65.4)	18 (64.3)	14 (53.8)
Failure to 1st line treatment				
	Yes	8 (30.8)	8 (28.6)	9 (34.6)
	No	18 (69.2)	20 (71.4)	17 (65.4)
Time from end of 1st line to relapse (mo)	n	26	28	26
	Mean (SD)	9.6 (11.5)	12.8 (17.4)	8.1 (11.8)
	Median	6.5	6.0	1.1
	(range)	(0 - 43.5)	(0 - 62.7)	(0 - 36.3)
Time since primary diagnosis	n	26	28	26
	Mean (SD)	32.1 (17.3)	36.4 (23.6)	28.0 (21.9)
	Median	33.7	32.0	23.4
	(range)	(3.7-67.7)	(3.9- 94.1)	(3.8- 71.4)
Time from primary diagnosis to relapse, very early (mo) [1]	**n**	**7 (26.9)**	**7 (25.0)**	**12 (46.2)**
	Mean (SD)	10.3 (5.7)	10.5 (5.1)	8.5 (5.1)
	Median	12.2	8.4	6.5
	(range)	3.7- 17.7	3.9- 17.1	3.8- 18.0
Time from primary diagnosis to relapse, early (mo) [2]	**n**	**6 (23.1)**	**8 (28.6)**	**5 (19.2)**
	Mean (SD)	30.7 (6.3)	27.2 (7.0)	30.5 (7.1)
	Median	30.0	26.0	31.3
	(range)	21.1-40.2	19.1-40.4	21.4-37.9
Time from primary diagnosis to relapse, late (mo) [2]	**n**	**13 (50.0)**	**13 (46.4)**	**9 (34.6)**
	Mean (SD)	44.6 (11.9)	56.1 (18.7)	52.8 (13.3)
	Median	38.9	50.7	51.4
	(range)	31.4- 67.7	31.2- 94.1	36.1-71.4
Site of relapse	Medullary	13 (50.0)	21 (75.0)	18 (69.2)
	Extramedullary+ medullary	7 (26.9)	3 (10.7)	6 (23.1)
	CNS	4 (15.4)	2 (7.1)	2 (7.7)
	Others	2 (7.7)	2 (7.1)	0
Risk groups	S1	2 (7.7)	3 (10.7)	0
	S2	14 (53.8)	12 (42.9)	12 (46.2)
	S3	1 (3.8)	6 (21.4)	0
	S4	9 (34.6)	7 (25)	14 (53.8)

N corresponds to the number of patients in treatment groups
Decimal digits were rounded to one decimal point for mean, median and SD.
[1] Classification of relapse is according to IBFM study group: a) very early relapse: time to relapse is <18 months after primary diagnosis; b) early relapse: more than 18 months after primary diagnosis and less than 6 months after first-line treatment ended; and c) late relapse: 6 months or more after first-line treatment ended.
Source: Table 14.1.9, Listing Appendix 16.2.4

Concomitant Medications

The reported concomitant medications were generally similar between the two treatment groups. Of note, the proportion of patients who received any anti-thrombotic agent, particularly antithrombin III, was significantly higher in the L-ASP arm (78.6%) compared to ERY001 arm (30.8%). In particular, antithrombin III was used in 5 (19.2%) patients and 17 (60.7%) patients.

An anti-diabetic treatment (insulin) was used in 2 patients (7.7%) and 6 patients (21.4%) in ERY001 and L-ASP arms, respectively.

In the single ERY001 arm, 11 patients (42.3%) received antithrombotic therapy, which was mainly anti-thrombin III. 50% of the patients received mainly another asparaginase following treatment with ERY001.

Table 7-8 Concomitant medication in at least 20% of patients - Treated analysis set

Concomitant medications [1]	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Antibacterials	26 (100)	28 (100)	26 (100)
Platelets	23 (88.5)	27 (96.4)	25 (96.2)
Antiemetics	24 (92.3)	27 (96.4)	24 (92.3)
Paracetamol	25 (96.2)	25 (89.3)	19 (73.1)
Red blood cells	21 (80.8)	23 (82.1)	21 (80.8)
Opioid analgesics			
Antithrombotic agents	8 (30.8)	22 (78.6)	11 (42.3)
Antithrombin III throughout study	5 (19.2)	17 (60.7)	4 (15.4)
Antacids and Proton pump inhibitors	15 (57.7)	22 (78.6)	21 (80.8)
Antimycotics	19 (73.1)	19 (67.9)	18 (69.2)
Laxatives	16 (61.5)	19 (67.9)	10 (38.5)
Corticosteroids	13 (50)	19 (67.9)	12 (46.2)
Antihistamines [2]	14 (53.8)	18 (64.3)	18 (69.2)
Spasfon (antispasmodic)	10 (38.5)	15 (53.6)	13 (50)
Diuretics	11 (42.3)	13 (46.4)	11 (42.3)
Antivirals	13 (50)	12 (42.9)	16 (61.5)
Albumin	4 (15.4)	11 (39.3)	9 (34.6)
Granulocyte colony stimulating factor	9 (34.6)	10 (35.7)	13 (50)
Allopurinol	4 (15.4)	6 (21.4)	4 (15.4)
Anti-diabetic treatment (Insulin)	2 (7.7)	6 (21.4)	2 (7.7)
Alprazolam	5 (19.2)	4 (14.3)	7 (26.9)

N corresponds to the number of patients in treatment groups
[1] Concomitant medication were recorded from inclusion date to the end of study date, as well as any concomitant medication reported in Adverse Events treated with a concomitant treatment
[2] Anti-histaminics were used either prophylactically or for active treatment of allergic reactions.
Source: Table 14.1.20. , Listing Appendix 16.2.4
Additional information on concomitant medication after treatment period is provided in Table 14.1.22.

Table 7-9 Anti-cancer therapy - Treated analysis set

Concomitant medications	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Throughout the study [1]	**12 (46.2)**	**14 (50)**	**14 (53.8)**
Asparaginase	7 (26.9)	11 (39.3)	11 (42.3)
Pegaspargase	5 (19.2)	6 (21.4)	5 (19.2)
Clofarabine	1 (3.8)	0	1 (3.8)
Cyclophosphamide	1 (3.8)	0	1 (3.8)
Fludarabine	0	0	1 (3.8)
Subsequent therapy following treatment with study medication	**12 (46.2)**	**12 (42.9)**	**13 (50)**
Asparaginase	7 (26.9)	9 (32.1)	10 (38.5)

	ERY001	L-ASP	ERY001-s
Pegaspargase	5 (19.2)	6 (21.4)	5 (19.2)
Clofarabine	1 (3.8)	0	1 (3.8)
Cyclophosphamide	1 (3.8)	0	1 (3.8)
Fludarabine	0	0	1 (3.8)

N corresponds to the number of patients in treatment groups
[1] Concomitant medication were recorded from inclusion date to the end of study date, as well as any concomitant medication reported in Adverse Events treated with a concomitant treatment
Source: Table 14.1.20, and Table 14.1.22.

Co-Primary endpoints

Mean duration of asparaginase activity >100IU/L

Table 8-1 Mean duration of asparaginase level >100 IU/L in whole blood during induction (days)– Treated analysis set

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
At least one day with asparaginase level >100 IU/L	No	0	1 (3.6)	0
	Yes	26 (100)	27 (96.4)	26 (100)
Duration of asparaginase level >100 IU/L				
	Mean (SD)	20.5 (5.2)	9.4 (7.4)	18.6 (6.3)
	CI (95%)	[18.36; 22.58]	[6.48; 12.23]	[16.04; 21.13]
	Median	22.0	7.2	21.8
	Min; Max	9.8; 30.7	0.0; 25.0	6.9; 27.9
Mean duration before 1st DSMB	N	10	10	11
At least one day with asparaginase level > 100 IU/L	Yes	10 (100)	10 (100)	11 (100)
Duration of asparaginase level > 100 IU/L				
	Mean (SD)	21.1 (4.3)	6.8 (5.2)	21.1 (4.4)
	CI (95%)	[18.08; 24.20]	[3.12; 10.50]	[18.14; 24.06]
	Median	22.1	6.7	21.9
	Min ; Max	9.8; 24.1	1.0; 18.9	10.0; 25.0
Mean duration between 1st and 2nd DSMB	N	12	12	10
At least one day with asparaginase level >100 IU/L	No	0	1 (8.3)	0
	Yes	12 (100)	11 (91.7)	10 (100)
Duration of asparaginase level >100 IU/L				
	Mean (SD)	19.5 (6.6)	9.6 (8.0)	15.1 (6.8)
	CI (95%)	[15.27; 23.69]	[4.51; 14.62]	[10.19; 19.96]
	Median	22.0	7.1	12.9
	Min; Max	10.0; 30.7	0.0; 25.0	6.9; 25.1
Mean duration following 2nd DSMB	N	4	6	5
At least one day with asparaginase level >100 IU/L	Yes	4 (100)	6 (100)	5 (100)
Duration of asparaginase level >100 IU/L				
	Mean (SD)	21.8 (2.0)	13.2 (8.9)	20.1 (6.6)
	CI (95%)	[18.54; 24.99]	[3.89; 22.47]	[11.89; 28.26]
	Median	21.99	11.45	20.99
	Min; Max	19.1; 24.0	4.0; 23.9	9.7; 27.9

N corresponds to the number of patients in treatment groups
The minimal BLLQ value corresponds to 75 IU in whole blood
Source Table: Table 14.4.1.1, Table 14.4.1.1.1, Table 14.4.1.1.2, Table 14.4.1.1.3

Table 8-2 Ratio of the mean of duration of asparaginase level >100 IU/L in whole blood during induction in the non-allergic randomized arms – Treated analysis set

Population	ERY001 N*	L-ASP N*	Mean Ratio	P-value for non-inferiority
Adaptive design [1]				
Entire study period	26	28	2.28	<0.001**
Standard deviation			0.37	
95% CI [a] [2]			1.55; 3.01	
95% CI [a] [3]			1.67; 3.07	
Patients in 1st DSMB	10	10	3.21	<0.001**
New patients in 2nd DSMB	12	12	2.17	<0.001**
New patients after 2nd DSMB	4	6	1.68	<0.001**
Fisher combination test#				<0.001
Analysis of variance [4]				
Estimate (95% CI)			11.02 [7.5; 14.6]	
LS Means (95% CI)	20.3 [17.7; 22.9]	9.3 [6.8; 11.8]		
P value for superiority			<0.001	

N corresponds to the number of patients in treatment groups
[1] Adaptive design, Bootstrap method – test for non-inferiority
[2] Method 1: 95% CI = [mean – 1.96 x standard deviation; mean + 1.96 x standard deviation);
[3] Method 2: 95% CI = [2.5 percentile of the distribution of the ratios from 1000 bootstrap samples ; 97.5 percentile of the distribution of the ratios from 1000 bootstrap samples]
[4] Analysis of Variance – test for superiority
The minimal BLLQ value corresponds to 75 IU in whole blood
[a] 95% Confidence interval was evaluated by two methods:
* N corresponds to the number of patients in treatment groups used to performed the bootstrap method with replacement
** Non-inferiority P-value was evaluated using z-score ((ratio of the mean - 0.8)/standard deviation) compared to the standard normal distribution
Mean and standard deviation ratio were evaluated using a bootstrap method with replacement
P-value is evaluated with the Fisher combination test according to the p-values evaluated for the 3 parts of the trial: prior the 1st interim evaluation by the DSMB, following that interim but prior to the 2nd interim DSMB and finally following the 2nd DSMB
Source:, Table 14.2.1.2.8, Table 14.2.1.2.9, Table 14.4.1.7

The mean duration of asparaginase >100 IU/L measured in whole blood was significantly higher in ERY001 arm compared to L-ASP arm, with a mean of 20.5 (SD: 5.2) days and 9.4 (SD: 7.4) days, respectively. Only one patient in L-ASP arm did not reach this threshold.

The ratio of the mean values (ERY001/L-ASP) was equal to 2.28. The 95% bootstrap confidence interval for this ratio is (1.55; 3.01). This lies completely above the non-inferiority margin of 0.8 and

the one-sided p-value for non-inferiority is <0.001. This 95% confidence interval is also completely above 1 providing evidence of the superiority of ERY001 over L-ASP for this endpoint. The ANOVA comparison of the means, adjusting for disease severity, between the treatment groups shows a highly statistically significant difference (p < 0.001) in favour of the ERY001 treatment.

In the analysis respecting the adaptive nature of the trial design, the mean duration of asparaginase activity levels >100 IU/L during induction for those recruited prior to the first DSMB meeting were 21.1 days (SD: 4.3) and 6.8 days (SD: 5.2); for patients recruited between the first DSMB meeting and the second were 19.5 days (SD: 6.6) and 9.6 days (SD: 8.0) days; and finally for those recruited after the second DSMB meeting were 21.8 days (SD: 2.0) and 13.2 days (SD: 8.9), for ERY001 and L-ASP arms, respectively, Table 8-1. The p-value for Fishers combination test is < 0.001, concluding that there is no evidence of an inconsistent treatment difference across the 3 parts of the trial.

Mean duration of asparaginase level in subgroups

The trend of prolonged asparaginase activity with ERY001 compared to L-ASP was maintained across all subsets. However, there are some observations that also require highlighting. Firstly, the mean duration of asparaginase activity >100 IU/L in adult patients treated with L-ASP was 3.2 days (SD: 2.8), which is greatly lower than observed in the entire L-ASP group. Secondly, although asparaginase level was greatly higher in the ERY001 (mean: 14.17 days; SD: 5.1) compared to L-ASP (mean: 6.5 days; SD: 4.0) in the subset of patients with positive antibody status at baseline, these levels were generally lower, as when compared to patients with negative antibody status at baseline.

The ratios of the mean values in all of the subgroups considered were greater than 1 ranging from 1.68 for children younger than 11 years to 6.24 for adults (≥18 years old) indicating homogeneity across subgroups in terms of ERY001 being numerically superior to L-ASP on average.

Table 8-3 Mean duration of total asparaginase level >100 IU/L in whole blood during induction in the non-allergic randomized arms adjusted for subgroups – Treated analysis set

	ERY001	L-ASP	ERY001	L-ASP
Age group	**Children**		**Adults**	
N	21	21	5	7
Mean (SD)	20.8 (5.3)	11.4 (7.3)	19.3 (5.5)	3.2 (2.8)
Median	22.0	8.3	22.1	2.3
Min; Max	10.0; 30.7	1.0; 25.0	9.8; 22.9	0.0; 9.0
Risk Score	**S1/S2**		**S3/S4**	
N	16	15	10	13
Mean (SD)	20.2 (6.0)	12.0 (8.6)	20.9 (3.9)	6.3 (4.4)
Median	22.4	9.0	22.0	6.2
Min ; Max	10.0; 30.7	1; 25.0	9.8; 22.9	0.0; 14.9
F1-F2/VANDA	**F1-F2**		**VANDA**	
N	16	15	10	13
Mean (SD)	19.5 ± 6.5	11.5 (9.0)	22.0 (0.2)	6.9 (4.2)
Median	22.9	8.8	22.0	6.9
Min; Max	9.8; 30.7	1.0; 25.0	21.8; 22.6	0.0; 14.9
Antibody status	**Negative**		**Positive**	
N	20	21	6	7
Mean (SD)	22.4 (3.6)	10.31 (8.1)	14.17 (5.1)	6.5 (4.0)
Median	22.4	8.3	12.2	7.0
Min; Max	10.2; 30.7	0.0; 25.0	9.8; 21.8	1.9; 14.0

N corresponds to the number of patients in each treatment subsets
Minimal BLLQ value in whole blood corresponds to 75 IU/L
Source: Table 14.4.1.2, Table 14.4.1.4, Table 14.4.1.5, Table 14.4.1.6,

Table 8-5 Mean duration of total asparaginase level >100 IU/L in whole blood during induction in population subsets treated with ERY001s – Treated analysis set

Age group	Children	Adults
N	15	11
Mean (SD)	18.2 (7.1)	19.1 (5.2)
Median	21.8	21.7
Min; Max	6.9; 27.9	9.1; 24.9
Risk Score	**S1/S2**	**S3/S4**
N	12	14
Mean (SD)	18.5 (7.6)	18.7 (5.2)
Median	21.5	21.8
Min; Max	6.9; 27.9	9.1; 22.9
F1-F2/VANDA	**F1-F2**	**VANDA**
N	12	14
Mean (SD)	17.4 (7.9)	19.6 (4.6)
Median	18.4	21.9
Min ; Max	6.9; 27.9	9.1; 22.9
Antibody status	**Negative**	**Positive**
N	11	15
Mean (SD)	18.9 (6.8)	18.4 (6.2)
Median	21.8	21.7
Min ; Max	9.1; 27.9	6.9; 25.0

N corresponds to the number of patients in each treatment subset
Source: Table 14.4.1.2, Table 14.4.1.4, Table 14.4.1.5, Table 14.4.1.6

Sensitivity Analyses

Mean duration of asparaginase level >75 IU/L and >400 IU/L

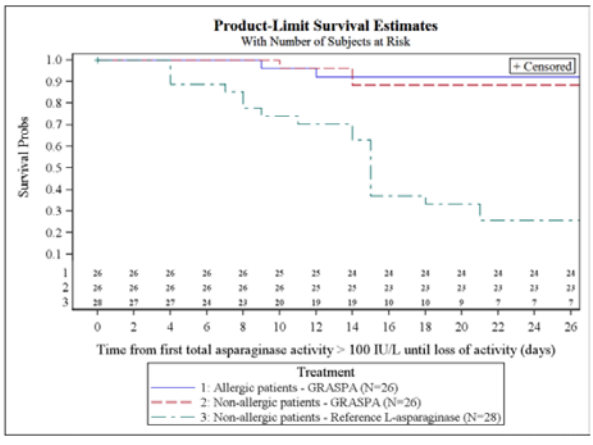
All patients in ERY001 arm and 27 (96.4%) patients in the L-ASP had levels above 75 IU/L, with a mean duration of 20.6 days (SD: 5.2) and 9.8 days (SD: 7.3), respectively; p value <0.001.

The asparaginase activity level >400 IU/L were achieved in all patients in the ERY001 arm, compared to 24 (85.7%) in L-ASP arm, with a mean duration of 18.6 days (SD: 7.0) and 4.7 days (SD: 5.5), respectively; p value <0.001,

Time to loss of total asparaginase activity >100 IU/L

The activity was retained in the majority of patients in the ERY001 arm (88.5%) compared to only 25.9% in the L-ASP arm. The median times from first assessment of activity >100 IU/L to loss of activity was not reached in the ERY001 arm by Day 28, but was 15 days in the L-ASP arm.

Figure 8-1 Kaplan-Meier plot of time from first assessment of asparaginase activity >100 IU/L until loss of that activity - Treated analysis set



Source Figure 14.4.6.2

Incidence Of Hypersensitivity Reactions During Induction

Table 8-12 Number (%) of patients who had at least one allergic reaction related to study treatment during induction (28 days) by preferred term – Treated analysis set

Preferred Term	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N= 26 (%)
At least one allergic reaction	**0**	**13 (46.4)**	**3 (11.5)**
Drug hypersensitivity	0	12 (42.9)	0
Anaphylactic reaction	0	1 (3.6)	0
Anaphylactic shock	0	1 (3.6)	0
Hypersensitivity	0	1 (3.6)	0
Erythema multiform	0	1 (3.6)	0
Facial edema	0	0	1 (3.8)
Edema	0	0	1 (3.8)
Allergic dermatitis	0	0	1 (3.8)
Difference of proportion (SD)	- 0.46 (0.09)		
95% CI*	- 0.69; -0.24		
P-value	<0.001#		

N corresponds to the number of patients in treatment groups
For each system organ class and preferred term patients are included only once even if they experienced multiple events in that system organ class or Preferred Term
P-value indicates if there is a significant difference for the occurrence of allergic reaction between ERY001 treatment arm and L-ASP arm / Evaluated with CMH test
* CI: Confidence Interval
Source: Table 14.2.1.4.1.4, Table 14.2.1.4.7.3

Table 8-13 Difference in proportions of patients suffering allergic reactions related to study treatment during induction phase (28 days) – Treated analysis set

Population	At least one hypersensitivity reaction		Difference in proportion (SD)	95% CI
	ERY001 (n/N)*	L-ASP (n/N)		
Adults ≥ 18 years	0/5	3/7	-0.43 (0.19)	[-0.97; 0.11]
Children <18 years	0/21	10/21	-0.48 (0.11)	[-0.74; -0.21]
F1-F2 induction	0/16	9/15	-0.60 (0.13)	[-0.91; -0.29]
VANDA induction	0/10	4/13	-0.31 (0.13)	[-0.65; 0.03]
Negative antibody status	0/20	7/21	-0.33 (0.10)	[-0.58; -0.08]
Positive antibody status	0/6	6/7	-0.86 (0.13)	[-1.27; -0.44]
Risk scoring S1-S2	0/16	8/15	-0.53 (0.13)	[-0.85; -0.22]
Risk scoring S3-S4	0/10	5/13	-0.38 (0.13)	[-0.74; -0.03]

* n = number of patients with hypersensitivity event; N= total number of patients in respective subgroup for each treatment arm

Source Table: 14.2.1.4.7.3

The difference in proportions in all of the subgroups was also less than 1 ranging from -0.31 (95% CI:(-0.65; 0.03)) for the high risk patients treated with VANDA induction to -0.86 (95% CI: (-1.27; -0.44)) for patients with positive antibody status at baseline, indicating that ERY001 is numerically superior to L-ASP.

Single ERY001 arm - Hypersensitivity reactions that occurred in 3 (11.5%) of patients treated with ERY001 during induction and were considered related to study drug.

Sensitivity Analyses Of The Co-Primary Safety Endpoint

The first sensitivity analysis took account of all allergic events during the induction period up to switch to another asparaginase, irrespective of relationship to treatment with study drug. The observed difference in proportions is -0.38 (95% CI: (-0.64, -0.13)), which was statistically significant (p = 0.002).

The second sensitivity analysis was more conservative and looked at the full 28 day period from start of treatment and counted all allergic events, and therefore, included all events related to study treatments and also all other events occurring following discontinuation of study treatment.

The conclusion, therefore, remain unchanged regarding the superiority of ERY001 over L-ASP in terms of reducing the incidence of related hypersensitivity reactions.

Table 8-14 Number (%) of patients who had at least one allergic reaction by preferred term regardless of relationship to treatment - Treated analysis set

Preferred Term	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
At least one allergy	**3 (11.5)**	**14 (50.0)**	**10 (38.5)**
Drug hypersensitivity	1 (3.8)	10 (35.7)	8 (30.8)
Hypersensitivity	0	1 (3.6)	1 (3.8)
Anaphylactic reaction	0	1 (3.6)	0
Anaphylactic shock	0	1 (3.6)	0
Food allergy	0	1 (3.6)	0
Angioedema	1 (3.8)	0	0
Dermatitis allergic	0	0	1 (3.8)
Erythema multiform	0	1 (3.6)	0
Allergic transfusion reaction	1 (3.8)	1 (3.6)	0
Face edema	0	0	1 (3.8)
Edema	0	0	1 (3.8)
Difference of proportion (SD)	-0.38 (0.11)		
95% CI*	-0.64; -0.13		
P-value	0.002		

N corresponds to the number of patients in treatment groups
* CI: Confidence Interval
Source: Table 14.2.1.4.8, Table 14.2.1.4.13.1

Secondary efficacy endpoints

Cytologic And Complete Remission Rate

At the end of induction, patients in ERY001 achieved a higher CR (65.4%, 95% CI: (51.6; 89.8)) as compared to L-ASP patients (39.3%, 95% CI: (23.3; 63.1)). The CR difference was statistically significant (p=0.026). The difference in the CR rate between the treatment arms was not statistically significant in the subgroup analysed, notably children or adult age groups, and antibody status at baseline.

Table 8-20 Summary of cytologic and complete remission rates at the end of F1-F2/VANDA and in subgroups– Treated analysis set

	ERY001	L-ASP	ERY001-s
F1-F2/VANDA			
Cytologic remission (N)	**26**	**28**	**26**
Missing values	3 (11.5)	3 (10.7)	2 (7.7)
No	2 (7.7)	11 (39.3)	9 (34.6)
Yes	21 (80.8)	14 (50.0)	15 (57.7)
Complete remission (N)	**26**	**28**	**26**
Missing values	3 (11.5)	2 (7.1)	2 (7.7)
No	6 (23.1)	15 (53.6)	10 (38.5)
95% CI	[10.2; 48.4]	[36.9; 76.7]	[22.1; 63.4]
Yes	17 (65.4%)	11 (39.3)	14 (53.8)
95% CI	[51.6; 89.8]	[23.3; 63.1]	[36.6; 77.9]
P Value		0.026	
CR rate at induction by age groups			
Children (N)	**21**	**21**	**15**
Missing values	3 (14.3)	2 (9.5)	0
No	4 (19.0)	10 (47.6)	4 (26.7)
Yes	14 (66.7)	9 (42.9)	11 (73.3)
P value		0.057*	
Adults (N)	**5**	**7**	**11**
Missing values	0	0	2 (18.2)
No	2 (40.0)	5 (71.4)	6 (54.5)
Yes	3 (60.0)	2 (28.6)	3 (27.3)
P value		0.558**	
CR rate at induction by antibody status			
Negative antibody status (N)	**20**	**21**	**11**
Missing values	1 (5.0)	1 (4.8)	1 (9.1)
No	4 (20.0)	10 (47.6)	3 (27.3)

	ERY001	L-ASP	ERY001-s
Yes	15 (75.0)	10 (47.6)	7 (63.6)
P value		0.057*	
Positive antibody status (N)	6	7	15
Missing values	2 (33.3)	1 (14.3)	1 (6.7)
No	2 (33.3)	5 (71.4)	7 (46.7)
Yes	2 (33.3)	1 (14.3)	7 (46.7)
P value		0.558**	

N corresponds to the number of patients in treatment subsets
* Comparison between ERY001 treatment group and L-ASP treatment group. P-value, Pearson Chi² test (Two-sided) because all expected cell count > 5
** Comparison between ERY001 treatment group and L-ASP treatment group. P-value, Fisher test (Two-sided) because at least one expected cell count ≤5
Source: Table 14.2.2.5.1, Table 14.2.2.5.2, Table 14.2.2.5.4

Allograft Procedure

The allograft rate was not a pre-planned endpoint in the study. However, there were numerically higher number of patients who successfully proceeded to graft procedure in the ERY001 arm (n=17 (65.4%) patients), compared to the L-ASP arm (n= 13 (46.4%). Median time to allograft for patients who achieved a complete remission was 4.4 months (95% CI: 3.4; 6.4) in ERY001 arm vs. 5.8 months (95% CI: 3.6) in L-ASP arm.

Minimal Residual Disease

The proportion of patients who achieved MRD $<10^{-3}$ following induction (F1-F2/VANDA) was similar in both treatment arms. The difference was not statistically significant (p =0.605),

Table 8-22 Summary of minimal residual disease following induction – Treated analysis set

	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
F1 - F2 / VANDA	**26**	**28**	**26**
Missing values	6	9	9
$<10^{-3}$	9 (34.6)	7 (25.0)	7 (26.9)
$\geq 10^{-3}$	11 (42.3)	12 (42.9)	10 (38.5)
P value*		0.605	
MRD absolute value	**26**	**28**	**26**
Missing values	7	12	11
$\geq 10^{-2}$	3 (11.5)	6 (21.4)	5 (19.2)
$\geq 10^{-3}$ and $<10^{-2}$	8 (30.8)	5 (17.9)	5 (19.2)
$\geq 10^{-4}$ and $<10^{-3}$	4 (15.4)	1 (3.6)	1 (3.8)
$\geq 10^{-5}$ and $<10^{-4}$	4 (15.4)	4 (14.3)	4 (15.4)

N corresponds to the number of patients in treatment groups;
* Comparison between ERY001 and L-ASP arms. P-value, Pearson Chi² test (Two-sided) because all expected cell count > 5
Source Table 14.2.2.6.1

Relapse, Event-Free Survival Rates, And Overall Survival

As expected, the relapse rate at 6 and 12 months was low. Except for L-ASP, and adult patients, the median EFS and OS was not reached for ERY001 in the entire set or in children, either at 6 or 12 months. Overall, there was a trend across all groups with lower EFS and OS event rates with ERY001 compared to L-ASP. However, none was statistically significant.

Table 8-23 Summary of relapse at 6 and 12 months – Treated analysis set

	ERY001 N=26 (%); 95% CI	L-ASP N=28 (%); 95% CI	ERY001-s N=26 (%); 95% CI
6 mo relapse rate	24	21	17
No	20 (83.3)	18 (85.7)	15 (88.2)
95% CI	[62.6; 95.3]	[63.7; 97.0]	[63.6; 98.5]
Yes	3 (12.5)	1 (4.8)	2 (11.8)
	[2.6; 32.4]	[0.1; 23.8]	[1.5; 36.4]
NA*	1 (4.2)	2 (9.5)	0
	[0.1; 21.1]	[1.2; 30.4]	
12 mo relapse rate	19	18	13
No	14 (73.7)	13 (72.2)	13 (100.0)
	[48.8; 90.9]	[46.5; 90.3]	[75.3; .]
Yes	5 (26.3)	3 (16.7)	0
	[9.1; 51.2]	[3.6; 41.4]	
NA**	0	2 (11.1)	0
		[1.4; 34.7]	

N corresponds to the number of patients in treatment groups
* At 6 months, relapses were reported as NA by the investigator for 3 patients:
- Patient #102-01 who received allograft between 6-months CR and end of the study;
- Patient #120-02 who had progressive disease at the end of treatment and did receive a graft at 6-months;
- Patient #125-11 who never had an interim CR.
** At 12 months, 2 patients were assessed as NA:
- Patient #101-03 who received a graft before 6 months, and met CR criteria at 12 months;
- Patient #102-01 who was still in CR at 12 months.
Source Table 14.2.2.7.1

Table 8-24 Summary of event-free survival and overall survival at 6 and 12 months in randomized treatment arms – Treated analysis set

	ERY001 vs. L-ASP		
	All patients ERY001, N = 26 L-ASP, N= 28	Children ERY001, N= 21 L-ASP, N= 21	Adults ERY001, N= 5 L-ASP, N= 7
6 mo EFS			
Median (mo)	NR	NR	4.6 vs. 1.6
Events	23.1% vs. 39.3%	14.3% vs. 28.6%	60% vs. 71.4%
HR	0.51	0.46	0.58
95% CI	0.19; 1.32	0.13. 1.72	0.14; 2.37
P Value*	0.164	0.251	0.451
12 mo EFS			
Median (mo)	NR vs. 11.6	NR	4.6 vs. 1.6
Events	30.8% vs. 50.0%	19.1% vs. 38.1%	80% vs. 85.7%
HR	0.54	0.47	0.69
95% CI	0.23; 1.26	0.15; 1.47	0.20; 2.44
P Value*	0.153	0.196	0.569
6 mo OS			
Median (mo)	NR	NR	NR
Events	7.7% vs. 21.4%	4.8% vs. 14.3%	20% vs. 42.9%
HR	0.35	0.34	0.39
95% CI	0.09; 1.39	0.05; 2.42	0.05; 2.81
P Value*	0.136	0.282	0.351
12 mo OS			
Median (mo)	NR	NR	11.2 vs. 8.2
Events	23.1% vs. 32.1%	4.8% vs. 14.3%	20% vs. 42.9%
HR	0.63	0.34	0.39
95% CI	0.23; 1.74	0.05; 2.42	0.05; 2.81
P Value*	0.377	0.424	0.705

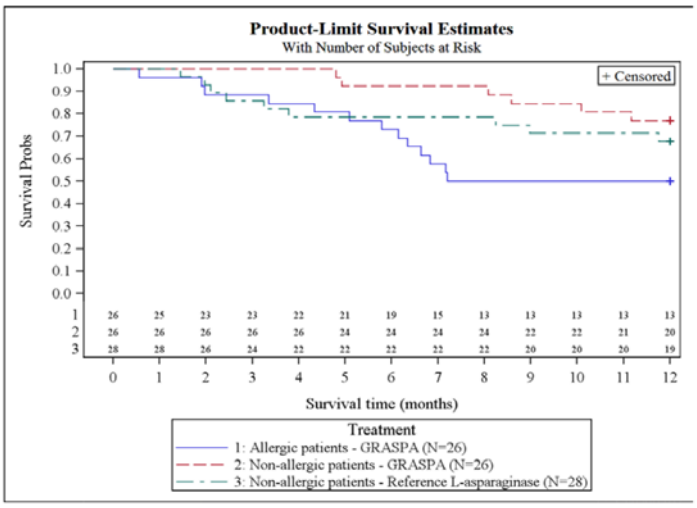
N corresponds to the number of patients in treatment groups
A Hazard Ratio greater than 1 will favor the reference (R). The analysis was performed using the Log Rank Test with treatment as the only factor (R) Reference for Hazard Ratio
NR: not reached
* P value in all subsets is not statistically significant
Source, Table 14.2.2.9.3, Table 14.2.2.9.3.1, Table 14.2.2.9.4, Table 14.2.2.9.4.1, Table 14.2.2.9.5, Table 14.2.2.9.5.1, Table 14.2.2.9.6, Table 14.2.2.9.6.1, Table 14.2.2.11.2, Table 14.2.2.11.2.1, Table 14.2.2.11.3, Table 14.2.2.11.3.1, Table 14.2.2.11.4, Table 14.2.2.11.4.1, Table 14.2.2.11.5, Table 14.2.2.11.5.1

Table 8-25 Summary of event-free survival and overall survival at 6 and 12 months in ERY001-s arm – Treated analysis set

		ERY001-s		
		All patients N= 26	Children N= 15	Adults N= 11
6 mo EFS	Median (mo)	NR	NR	1
	Events	38.5%	20%	63.6%
12 mo EFS	Median (mo)	NR	NR	1
	Events	46.2%	26.7%	72.7%
6 mo OS	Median (mo)	NR	NR	NR
	Events	26.9%	13.3%	45.5%
12 mo OS	Median (mo)	NR	NR	6.2
	Events	50.0%	13.3%	45.5%

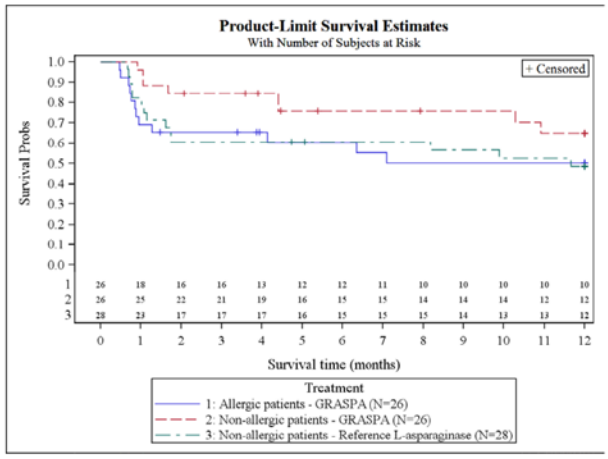
N corresponds to the number of patients in treatment groups
NR: not reached
Source Table 14.2.2.9.3, Table 14.2.2.9.3.1, Table 14.2.2.9.4, Table 14.2.2.9.4.1, Table 14.2.2.9.5, Table 14.2.2.9.5.1, Table 14.2.2.9.6, Table 14.2.2.9.6.1, Table 14.2.2.11.2, Table 14.2.2.11.3, Table 14.2.2.11.2.1, Table 14.2.2.11.3.1, Table 14.2.2.11.4.1, Table 14.2.2.11.5, Table 14.2.2.11.5.1

Figure 8-3 Kaplan-Meier plot of overall survival in all groups – Treated analysis set



Source Figure 14.2.2.9.1

Figure 8-4 Kaplan-Meier plot of event-free survival for all treatment groups – Treated analysis set



Source Figure 14.2.2.11.1

Asparagine depletion ≤2 μM during induction phase

The mean duration of asparagine depletion ≤2 μM was higher in L-ASP arm compared to ERY001 arm, with a mean of 12.3 (SD: 7.8) days and 7.1 (SD: 5.7) days, respectively. The maximum duration of asparagine depletion was 19 days and 25 days, in the ERY001 and L-ASP arms, respectively.

The ratio of the mean values (ERY001/L-ASP) was equal to 0.59. The 95% bootstrap confidence interval for this ratio is (0.38; 0.82). This crossed the non-inferiority margin of 0.8, and the one-sided p-value for non-inferiority is 1.000, indicating that the non-inferiority of ERY001 in terms of the mean duration of asparaginase depletion ≤2 μM has not been established.

Table 8-26 Mean duration of asparagine depletion ≤ 2 µM during induction phase (days) – Treated analysis set

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
At least one day with asparagine depletion ≤2 µM	No	6 (23.1)	1 (3.6)	11 (42.3)
	Yes	20 (76.9)	27 (96.4)	15 (57.7)
Duration of asparagine depletion ≤2 µM				
	Mean (SD)	7.1 (5.7)	12.3 (7.8)	2.4 (3.2)
	CI (95%)	[4.82 ; 9.44]	[9.28 ; 15.32]	[1.13 ; 3.75]
	Median	6.01	8.96	1.31
	Min; Max	0.0 ; 18.8	0.0 ; 25.0	0.0 ; 10.0
Mean duration before 1st DSMB	N	10	10	11
At least one day with asparagine depletion ≤2 µM	Yes	7 (70.0)	9 (90.0)	10 (90.9)
Duration of asparagine depletion ≤2 µM				
	Mean (SD)	5.9 (5.6)	8.2 (6.8)	3.3 (3.3)
	CI (95%)	[1.89; 9.95]	[3.38; 13.08]	[1.09; 5.52]
	Median	5.80	6.54	2.91
	Min; Max	0.0; 15.0	0.0; 25.0	0.0; 10.0
Mean duration between 1st and 2nd DSMB	N	12	12	10
At least one day with asparagine depletion ≤ 2 µM	No	3 (25.0)	0	6 (60.0)
	Yes	9 (75.0)	12 (100.0)	4 (40.0)
Duration of asparagine depletion ≤ 2 µM				
	Mean (SD)	7.4 (6.1)	13.7 (7.8)	1.8 (2.9)
	CI (95%)	[3.58; 11.30]	[8.78; 18.70]	[-0.28; 3.92]
	Median	8.30	12.04	0.00
	Min; Max	0.0; 18.8	2.3; 25.0	0.0; 9.1

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Mean duration following 2nd DSMB	N	4	6	5
At least one day with asparagine depletion ≤2 µM	Yes	4 (100)	6 (100)	1 (20.0)
Duration of asparagine depletion ≤2 µM				
	Mean (SD)	9.2 (5.7)	16.2 (7.4)	1.8 (4.0)
	CI (95%)	[0.19 ; 18.24]	[8.50 ; 23.94]	[-3.14 ; 6.67]
	Median	9.49	16.50	0.00
	Min; Max	2.9 ; 14.9	8.1 ; 23.9	0.0 ; 8.8

N corresponds to the number of patients in treatment groups
Source: Table 14.2.1.1.1, Table 14.2.1.1.1.1, Table 14.2.1.1.1.2, Table 14.2.1.1.1.3

Subset analyses

The trend of shorter duration of asparagine depletion with ERY001 compared to L-ASP was observed across all subsets except in the small adult patient subset. Also, of interest is the lack of asparagine depletion in patients with positive antibody status at baseline.

None of the patients in the ERY001 arm or L-ASP arm presented with both asparagine depletion and absence of allergic reactions in the F1/F2 standard risk. In the VANDA group, two patients in each arm presented with asparagine depletion and no evidence of allergic reactions.

Table 8-28 Mean duration of asparagine depletion ≤2 µM during induction in the non-allergic randomized subgroups – Treated analysis set

Age group	ERY001	L-ASP	ERY001	L-ASP
	Children		Adults	
N	21	21	5	7
Mean (SD)	7.0 (5.5)	13.8 (8.0)	7.7 (7.5)	7.8 (5.5)
Median	6.00	8.97	8.63	8.96
Min; Max	0.0; 18.8	4.0; 25.0	0.0; 15.0	0.0; 15.1
Risk Score	S1/S2		S3/S4	
N	16	15	10	13
Mean (SD)	6.6 (5.8)	12.9 (8.6)	7.9 (5.8)	11.6 (7.0)
Median	5.94	8.96	8.30	11.15
Min ; Max	0.0; 18.8	2.3; 25.0	0.0; 15.0	0.0; 22.1
F1-F2/VANDA	F1-F2		VANDA	
N	16	15	10	13
Mean (SD)	6.8 (6.3)	12.6 (8.8)	7.7 (5.0)	12.0 (6.7)
Median	5.94	8.95	8.30	12.02
Min; Max	0.0; 18.8	2.3; 25.0	0.0; 15.0	0.0; 22.1
Antibody status	Negative		Positive	
N	20	21	6	7
Mean (SD)	9.3 (4.7)	13.8 (7.9)	0	7.9 (6.0)
Median	8.70	12.02	0	7.02
Min; Max	2.9; 18.8	0.0; 25.0	0.0; 0.0	2.3; 21.0

N corresponds to the number of patients in treatment groups
Source: Table 14.2.1.1.2, Table 14.2.1.1.4, Table 14.2.1.1.5, Table 14.2.1.1.6

Table 8-30 Mean duration of asparagine depletion ≤2 µM during induction in in allergic patients subgroups treated with ERY001s – Treated analysis set

Age group	Children	Adults
N	15	11
Mean (SD)	2.9 (3.0)	1.9 (3.6)
Median	2.93	0.00
Min; Max	0.0; 10.0	0.0; 9.1
Risk Score	S1/S2	S3/S4
N	12	14
Mean (SD)	2.1 (2.5)	2.7 (3.8)
Median	1.93	0.37
Min; Max	0.0; 8.8	0.0; 10.0
F1-F2/VANDA	F1-F2	VANDA
N	12	14
Mean (SD)	1.8 (2.6)	3.0 (3.7)
Median	1.08	1.82
Min ; Max	0.0; 8.8	0.0; 10.0
Antibody status	Negative	Positive
N	11	15
Mean (SD)	5.2 (3.3)	0.4 (1.0)
Median	3.04	0.00
Min ; Max	2.0; 10.0	0.0; 3.7

N corresponds to the number of patients in treatment groups
Source: Table 14.2.1.1.2, Table 14.2.1.1.4, Table 14.2.1.1.5, Table 14.2.1.1.6

Sensitivity analyses

Sensitivity analyses based on cut-offs of ≤0.52 µM and ≤1 µM for asparagine depletion showed that the proportion of patients and mean duration for each level is similar to that observed with ≤2 µM. Although no formal statistical analysis was done for the ratio of the means, the conclusion appears unchanged, with the mean (95% CI) and median almost identical to that observed with asparagine depletion ≤2 µM.

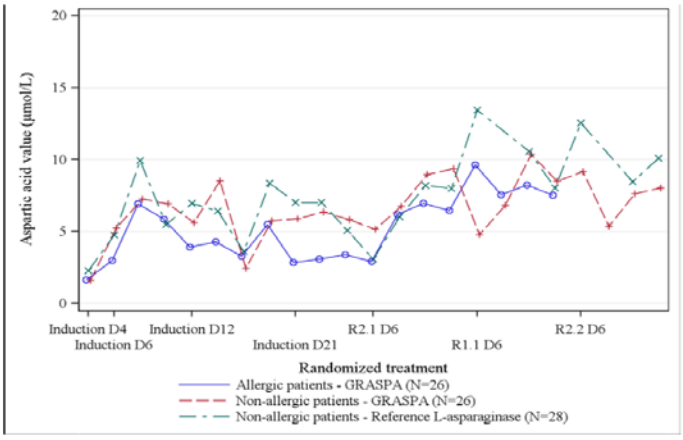
Amino Acid Changes (Aspartic acid, Glutamic acid and Glutamine)

Serum aspartic acid levels increased rapidly by Day 4, and remained elevated for ~3 weeks with ERY001 and L-ASP during induction.

Serum glutamine levels declined for the first 2 weeks of induction with ERY001 and L-ASP. Serum concentrations were slightly higher with ERY001.

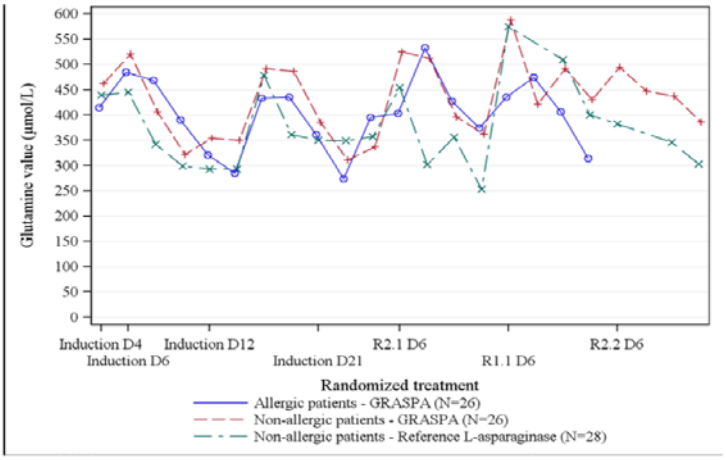
In L-ASP group, serum glutamic acid was moderately elevated with ERY001 compared to L-ASP,

Figure 8-7 Evolution of median aspartic acid over time – Treated analysis set



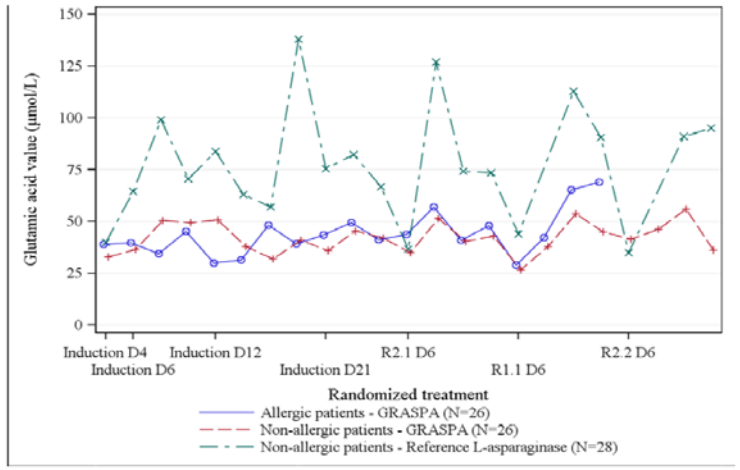
Note: If there are less than 5 patients in a group at a time point, data are not presented for this group
Outlier values of patient 120-03 at Induction-D9 (1223.1 µmol/L) and of patient 125-10 at Induction-D21 (357.2 µmol/L) were not take into account in the figure
Source: Figure 14.2.2.8.2, Table 14.2.2.8.2

Figure 8-8 Evolution of median glutamine over time – treated analysis set



Note: If there are less than 5 patients in a group at a time point, data are not presented for this group
Source: Figure 14.2.2.8.3, Table 14.2.2.8.3

Figure 8-9 Evolution of median glutamic acid over time – Treated analysis set



Note: If there are less than 5 patients in a group at a time point, data are not presented for this group
Outlier value of patient 120-03 at Induction-D9 (1986.3 µmol/L) was not taken into account in the figure
Source: Figure 14.2.2.8.4, Table 14.2.2.8.4

Summary of main efficacy results

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 1. Summary of efficacy for Study GraspaLL 2009-06

Title:		
Study identifier	Pivotal Phase II/III Study GraspaLL 2009-06.	
Design	This is a randomized open-label multicentre study to evaluate the efficacy and tolerability of Graspa (150 IU/Kg) compared to native L-asparaginase (L-ASP, 10,000 IU/Kg), each combined with COOPRALL chemotherapy. The study patients were children and adults with refractory or relapsed Ph- ALL, with or without evidence of prior hypersensitivity reactions.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	4 weeks 36 months (data provided 12 months FU)
Hypothesis	Non-inferiority with switch to superiority preplanned for the randomized cohorts. Exploratory for the non-randomized cohort	
Treatments groups	Graspa	150 IU/kg D4 and D18 of F1-F2 blocks or on D6 of VANDA block, then on D6 of R2/R1 blocks for up to 25 months
	L-asparaginase (native)	10000IU/m2 every 3 days starting on D4 F1 block (or D6 of VANDA block) for up to 24 months
	Graspas	150 IU/kg D4 and D18 of F1-F2 blocks or on D6 of VANDA block, then on D6 of R2/R1 blocks for up to 25 months

Endpoints and definitions	Co-Primary endpoint	-the mean duration (in days) of L-asparaginase activity >100 IU/L; and -the incidence of study drug-related allergic reactions during the induction phase.	Asparaginase activity was measured in plasma and whole blood. The primary analysis is based on whole blood in both treatment arms
	Secondary endpoint	CR MRD RR at 6m and 12m EFS 6m and 12m OS 6m and 12m Asparagine depletion <2µM Aminoacid changes (aspartic acid, bglutamic acid, glutamine) Allograft procedure Immunogenicity	
Database lock			

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	ITT			
Descriptive statistics and estimate variability	Treatment group	Graspa	L-asp	Graspas (allergic patients)
	Number of subject	26	28	26
	Duration of asparaginase activity >100 IU/L (mean)	20.5	9.5	18.6
	CI 95%	(18.36, 22.58)	(6.48, 12.23)	(16.04, 21.13)
	HS Reactions (proportion)	0%	46.4%	11.5%
	CI 95% not presented			
	Asparagine depletion <2µM during induction (Mean) <CI95%>	7.1 (4.82, 9.44)	12.3 (9.28, 15.32)	2.4 (1.13, 3.75)
Effect estimate per	Co-Primary	Comparison groups	Graspa vs L-asp	

comparison	endpoint Asparaginase activity	Ratio of means	2.28
		95% CI	1.55; 3.01
		P-value N-I P-value superiority	P<0.001 P<0.001
	Co-Primary HS reactions	Comparison groups	Graspa vs L-asp
		Differences in proportions	-0.46
		95% CI	(-0.69; 0.24)
		P-value	< 0.001
	Initially established primary endpoint (asparagine depletion <2µM	Comparison groups	Graspa vs L-asp
		Ratio of the mean values	0.59
		95% CI	(0.38; 0.82)
		Non-inferiority testing	Not demonstrated
Notes	For the Graspas cohort, data are just descriptive		

Clinical studies in special populations

The studies were conducted in children, adults and elderly patients. A description is just presented.

Table 2.7.3- 43 Impact of age on key efficacy analyses in non-allergic patients - Study GRASPALL 2009-06

	GRASPA	L-ASP	GRASPA	L-ASP
Age	Children		Adults	
Asparaginase activity				
N	21	21	5	7
Mean (SD)	20.8 (5.3)	11.4 (7.3)	19.3 (5.5)	3.2 (2.8)
Median	22.0	8.3	22.1	2.3
Min; Max	10.0; 30.7	1.0; 25.0	9.8; 22.9	0.0; 9.0
CR rate				
N	21	21	5	7
Yes	14 (66.7)	9 (42.9)	3 (60.0)	2 (28.6)
No	4 (19.0)	10 (47.6)	2 (40.0)	5 (71.4)
Missing value	3 (14.3)	2 (9.5)	0	0
1- year EFS				
N	21	21	5	7
Median	NR	NR	4.6	1.6
Events	19.1%	38.1%	80%	85.7%
HR (95% CI)	0.47 (0.15; 1.47)		0.69 (0.20; 2.44)	
1- year OS				
N	21	21	5	7
Median	NR	NR	11.2	8.2
Events	4.8%	14.3%	20%	42.9%
HR (95% CI)	0.34 (0.05; 2.42)		0.39 (0.05; 2.81)	
Duration of asparagine depletion ≤ 2 µM				
N	21	21	5	7
Mean (SD)	7.0 (5.5)	13.8 (8.0)	7.7 (7.5)	7.8 (5.5)
Median	6.00	8.97	8.63	8.96
Min; Max	0.0; 18.8	4.0; 25.0	0.0; 15.0	0.0; 15.1

NR: not reached
A Hazard Ratio greater than 1 will favor the reference (R). The analysis was performed using the Log Rank
Source: Tables: 8-3, Table 8-20, Table 8-24, 8-28

No dedicated studies have been conducted in patients with renal and/or hepatic impairment, which is considered justified. Appropriate information should be included in the SmPC.

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

N/A

Supportive studies

Study GraspaLL/ GRAALL SA2-2008

Study design and patient populations

This was a Phase IIa open-label study conducted in France evaluating the safety of Graspa in combination with multi-agent chemotherapy for the treatment of elderly patients aged 55 years or older. Eligible patients were males or females aged ≥ 55 years old, presenting with newly diagnosed Ph- ALL and ECOG PS score of 0,1 or 2. Patients who presented with relapsed disease or with prior exposure to L-asparaginase were excluded from the study.

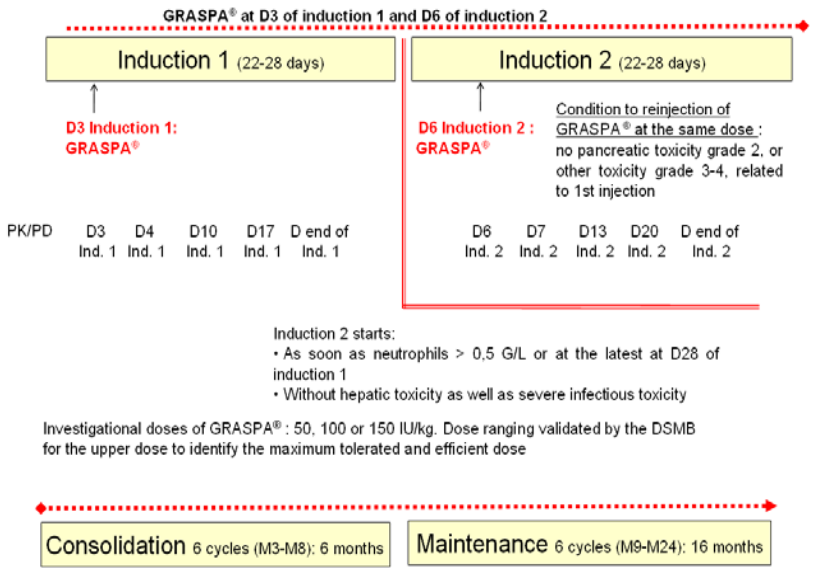
The primary objective of the study was to determine the maximal tolerated dose of Graspa in combination with chemotherapy. Additional objectives included safety and tolerability, evaluation of clinical activity, and pharmacokinetics and pharmacodynamics. The co-primary endpoints of the study were the duration of asparagine depletion ≤ 2 µM for at least 7 days after Graspa dosing, and the absence of dose-limiting toxicity.

Patients were treated with Graspa at escalating doses of 50, 100, and 150 IU/Kg, as a component of combination with the European Working group for Adult Lymphoblastic Leukemia (EWALL) chemotherapy protocol, which consisted of 2 induction blocks (Induction 1 and 2, each lasting 22-28 days), followed by 6 cycles of consolidation phase, and then 6 cycles of maintenance phase.

Treatment with Graspa was given on Day 3 of induction 1. If no toxicity was observed, then another dose of Graspa was administered on Day 6 of induction 2. Dose-limiting toxicities were defined as Grade 2 to Grade 4 pancreatic toxicity or other Grade 3 or Grade 4 toxicities during induction. Toxicity was defined as Grade 2 to 4 pancreatic toxicity or other Grade 3 or 4 toxicity during Induction 1 and Induction 2.

Patients were followed for 2 years, with regular safety assessments. A DSMB was established to monitor and review the safety of the patients following each dosing and advise on dose escalation.

The study design is depicted in Figure 2



Protocol amendments

There were 5 protocol amendments to the study protocol; all but one were done after the start of study recruitment. Three amendments were administrative only. The key changes are described below (more details of these amendments are presented in Section 9.8.1 of the CSR.

• Amendment 4 changed the product status from a blood product to an investigational medicinal product (IMP) status, following the request from AFSSAPS/ANSM.

• Amendment 5 added additional sampling for evaluation of anti-L-asparaginase antibodies.

Study endpoints

- Co-primary endpoints

The co-primary endpoints of the study were percentage of patients responding to treatment during Induction 1, i.e. with asparagine depletion $\leq$ 2 μM for a duration of at least 7 days after the administration of Graspa, and the presence of dose limiting toxicity (DLT) during Induction 1 or 2. Both co-primary endpoints are presented and discussed in this summary.

The DLTs were defined as:

• Grade 2 to 4 of pancreatic toxicity, Grade 3 or 4 hepatic toxicity, allergic reactions or deep cerebral thrombosis, potentially related to L-asparaginase,

• Hematological toxicity defined as bone marrow blast-free aplasia, 30 days following the last injection of chemotherapy,

• All other Grade 4 toxicities.

For asparagine depletion, levels were measured at the central laboratories, and were performed at Days 3, 4, and 10 of Induction 1, and Days 6, 7 and 13 of Induction 2. Only the depletion assessed at Day 10 of induction 1 was considered for the main efficacy criterion.

- Secondary efficacy endpoints

Pharmacodynamic Assessments: These included: plasma and CSF concentrations of amino acids (aspartate, glutamine, glutamate) during induction; free and encapsulated L-asparaginase activity; and specific anti-L-asparaginase antibody levels at baseline and during induction.

CR rate: Percentage of patients with CR at the end of Induction 1 and Induction 2 as assessed by the investigator, and was defined as absence of any physical evidence of leukaemia, evidence of normal CBC, and normal bone marrow with blasts <5%.

MRD rate: assessed during Induction 1 and Induction 2. The cut-off threshold for MRD negativity was defined as presence of leukemic cells $<10^{-4}$ in 2 assessments. If the two used MRD assessments led to different MRD levels, then the highest level was used.

Survival: survival status was assessed by the investigator 6-, 12-, and 24 months after the patient inclusion. The following parameters were evaluated:

• OS: Time from inclusion until death due to any cause;

• EFS: Time from inclusion until the first documented sign of disease relapse or death due to any cause.

• DFS: Time from inclusion until the first documented sign of disease relapse.

Statistical analysis

The analysis population included all patients enrolled into the study, who received at least one dose of the study drug.

Each cohort of patients included patients based on the intended dose for that cohort. There were 4 patients, who received 120 IU/Kg in induction 1, and were thus included in the 150 IU/Kg cohort.

Sample size considerations

The co-primary endpoints of the study were percentage of patients responding to treatment during Induction 1, i.e. with asparagine depletion $\leq$ 2 μM for a duration of at least 7 days after the administration of Graspa, and the presence of DLTs during Induction 1 or 2. The sample size in this study was based on a Bayesian adaptive design for which a minimum of 9 patients (3 patients for each dose) and a maximum of 30 patients were deemed sufficient as justified by statistical simulations.

The study was defined according to a dose escalation design, and the analyses were performed in several steps. The trial began by treating 3 patients with the lowest dose of 50 IU/kg. At each step, the choice of the following dose in the next cohort was based on the DSMB decisions.

Primary analysis

The Bayesian methodology was based on trade-offs between the probabilities of toxicity and efficacy of the treatment. At each dose level, these probabilities were updated, based on observations and information available during the study. The decision on the choice of the dose administered to the next cohort was based on these probabilities and on the recommendations of DSMB. The procedure was repeated until the maximum tolerated and efficient dose was identified, or the total number of patients was reached. For this adaptive design, a minimum of 9 patients and a maximum of 30 patients were deemed sufficient. The mathematical model used was the 2-variable Bayesian logistical regression: two-variable logistical regression consisted of two logistical regressions. The first regression calculated the probability of dose being toxic, the second logistical regression calculated the probability of dose being efficient.

The model suggested that the probabilities of a patient suffering from DLT and the probabilities of a patient responding to treatment increases with the dose (linear model).

Several interim analyses were performed due to the dose escalation design of the study. A DSMB was established to review safety and efficacy of treatment and recommend dose escalations.

Secondary efficacy analysis

For the secondary efficacy endpoints, summary descriptive statistics and 95% confidence intervals for treatment differences rather than formal inferences through p-values. P-values were presented where appropriate but any findings arising from such analyses were considered as supportive for the primary endpoints. For the time to event endpoints (for example OS and PFS) Kaplan-Meier curves were presented.

- **Study results**

Patient disposition

From March 30th, 2009, to October 19th, 2010, a total of 30 patients were enrolled in the study. A total of 3 (10.0%), 13 (43.3%) and 14 (46.7%) patients received Graspa at doses of 50 IU/Kg, 100 IU/Kg, and 150 IU/kg, respectively. A total of 4 patients received 120 IU/kg in Induction 1, because of overweight and/or the volume of the donor blood bag was too small in order to technically reach the dose of 150 IU/kg. These 4 patients were considered as receiving the intended dose of 150 IU/kg.

All patients received their first treatment with Graspa in Induction 1. A total of 24 patients (80.0%) started Induction 2: 23 patients (76.7%) received their second treatment with Graspa. The main reason for study discontinuation during induction 1 and 2 was death (6 (20%) patients). Four patients

died during Induction 1 (metabolic encephalopathy, arrhythmia, septic shock and cerebrovascular accident), and 2 patients during Induction 2 (tumor lysis syndrome and septic shock). The majority of patients died by 24 months follow-up.

Table 2.7.3- 12 Summary of patient disposition - Study GRASPALL/GRAALL SA2-2008

		50 IU/kg N=3	100 IU/kg N=13	150 IU/kg N=14
Received treatment	50 IU/kg	3 (100.0)	0	0
	100 IU/kg	0	13 (100.0)	0
	120 IU/kg	0	0	3 (21.4)[a]
	150 IU/kg	0	0	11 (78.6)
Received Induction 1	N	3 (100.0)	13 (100.0)	14 (100.0)
Study withdrawal during Induction 1	N	1		5
	Protocol change	0		1 (20.0)
	Death	1 (100.0)		3 (60.0)
	Other: Toxicity	0		1 (20.0)
Received Induction 2	N	2 (66.7)	13 (100.0)	9 (64.3)
Study withdrawal during Induction 2	N		3	1
	Death		2 (66.7)	0
	Treatment withdrawal		0	1 (100.0)
	Treatment failure		1 (33.3)	0
Study withdrawal at 24 mo	No	1 (33.3)	3 (23.1)	2 (14.3)
	Yes	2 (66.7)	10 (76.9)	12 (85.7)
Relapse	Yes	1 (33.3)	7 (53.8)	6 (42.9)
Death	Yes	2 (66.7)	10 (76.9)	9 (64.3)

There were 4 patients who received at least GRASPA at 120 IU/Kg. However, one of these patients received one dose at 120 IU/Kg, and one dose at 150 IU/Kg; according to the protocol, this patient was assigned to the 150 IU/Kg dose level

Protocol violations

Among the 30 patients included, 17 (56.7%) patients had at least one deviation. Major deviations accounted for 7 (23.3%) patients. The main deviations were related to deviations from the planned dose, dosing delays due to AEs, or prohibited medication.

Baseline demographics

The majority of the patients in this study were less than 75 years old, and presented with Performance Score of 0 or 2. The 3 cohorts were generally balanced with regards to gender, age groups, and Performance score, Table 2.7.3- 13.

Table 2.7.3- 13 Demographic characteristics- Study GRASPALL/ GRAALL SA2-2008

		Treatment dose Number (%)			
		50 IU/Kg N=3	100 IU/Kg N=13	150 IU/Kg N=14	Total N=30
Gender	N	3	13	14	30
	Male	1 (33.3)	7 (53.8)	6 (42.9)	14 (46.7)
	Female	2 (66.7)	6 (46.2)	8 (57.1)	16 (53.3)
Age (years)	N	3	13	14	30
	Mean ± SD	68.33 ± 3.51	66.92 ± 5.19	67.00 ± 5.56	67.10 ± 5.10
	Min ; Max	65.0; 72.0	59.0; 77.0	61.0; 77.0	59.0; 77.0
Age group (years)	N	3	13	14	30
	<65	0	5 (38.5)	7 (50.0)	12 (40.0)
	[65 ; 75[	3 (100.0%)	7 (53.8)	5 (35.7)	15 (50.0)
	≥ 75	0	1 (7.7)	2 (14.3)	3 (10.0)
ECOG PS	N	3	13	14	30
	0	3 (100.0)	3 (23.1)	3 (21.4)	9 (30.0)
	1	0	7 (53.8)	8 (57.1)	15 (50.0)
	2	0	3 (23.1)	3 (21.4)	6 (20.0)

Source: Table 7, and Table 8 of CSR

Baseline disease characteristics

Patients included in this study were newly diagnosed with ALL. The majority of the patients (86.7%) had B-cell ALL, without extramedullary disease.

Prior relevant medical history

The most frequent relevant medical history was: vascular disorders (26.7%), neoplasms (23.3%), and surgical and medical procedures (23.3%). The most frequent concomitant diseases were hypertension (56.7%), hypercholesterolemia (20.0%), type 2 diabetes mellitus (16.7%), dyslipidemia (10.0%), gamma-glutamyl increased (10.0%), anxiety (10.0%) , depression (10.0%) , osteoporosis (10.0%) and drug hypersensitivity (10.0%).

Concomitant medications

All patients of the analysis set received at least one concomitant medication during the study, mainly systemic antibacterial treatment (80%), blood substitutes and perfusion solutions (60%) and antithrombotic agents (mainly antithrombin III, 36.7%).

Primary efficacy analysis

A total of 21 (70%) patients achieved a asparagine depletion $\leq 2\ \mu M$ for at least 7 days at the end of induction, with 0%, 11 (84.6%), and 10 (71.4%) patients in the 50, 100, and 150 IU/kg dose levels, respectively. The mean asparagine depletion duration was higher in the 100 IU/Kg group (12.6 days; [range: 3.6-27.0 days]). Sensitivity analysis on time to depletion gave concordant results: A trend in favor of the dose of 100 IU/kg was observed compared with the dose of 150 IU/kg in terms of depletion duration (HR=1.8, 95% CI 0.8 - 4.0, p value = 0.18), Table 2.7.3- 32.

Dose limiting toxicities were reported in 2 and 4 patients in the 100 IU/Kg and 150 IU/Kg dose levels, respectively, and were mainly elevated liver and pancreatic enzymes, Table 2.7.3- 33.

The two criteria for efficacy and safety ("probability >70% than more than 67% of patients were efficient" and "probability >70% than less than 33% of patients suffered from DLT") were reached for the dose level of 150 IU/kg (probabilities of 90% and 78%, respectively).

According to the Bayesian statistical model, the maximum tolerated and efficient dose was, therefore, the highest dose, i.e. 150 IU/kg. However, based on the overall efficacy and safety results in induction and consolidation periods, the dose of 100 IU/kg was recommended for further studies in patient population. This dose level was effective with a high rate of asparagine responders (mean asparagine depletion of 12.6 days) and a high rate of clinical CR, and was well tolerated.

Table 2.7.3- 32 Summary of asparagine depletion according to plasma asparagine ≤ 2 µM- Study GRASPALL/GRALL SA2-2008

		Treatment Dose Number (%) of Patients		
		50 IU/kg N=3	100 IU/kg N=13	150 IU/kg N=14
Induction 1: Asparagine depletion ≤2 µM, for at least 7 days	N	3	13	14
	No	3 (100.0)	2 (15.4)	4 (28.6)
	Yes	0 (0.0)	11 (84.6)	10 (71.4)
	Mean ± SD	3.99 ± 0.02	12.55 ± 6.42	9.02 ± 3.72
	Median	3.99	10.48	10.44
	Min, Max	4.0, 4.0	3.6, 27.0	3.7, 16.5

Source: Table 12 of the CSR

Table 2.7.3- 33 Summary of efficacy and toxicity status - Study GRASPALL/GRALL SA2-2008

		Treatment Dose Number (%) of Patients		
		50 IU/kg N=3	100 IU/kg N=13	150 IU/kg N=14
Depletion for at least 7 days during Induction 1	Limiting toxicity occurred in Induction 1 and/or induction 2	0	2 (15.4)	3 (21.4)
	No limiting toxicity occurred in Induction 1 and/or induction 2	0	9 (69.2)	7 (50.0)
No depletion for at least 7 days during Induction 1	Limiting toxicity occurred in Induction 1 and/or induction 2	0	0	2 (14.3)
	No limiting toxicity occurred in Induction 1 and/or induction 2	3 (100.0)	2 (15.4)	2 (14.3)

Source: Table 15 of the CSR

Secondary efficacy endpoints

Total asparaginase activity >100 IU/L were achieved at all dose levels, with a trend toward higher levels at the 150 IU/kg of Graspa.

Clinical activity

CR and MRD rates were a key secondary endpoint in this study. Complete remission rate was 71.4% and 86.4%, at the end of Induction 1 and 2, respectively. The MRD rates <10-3 at the end of Induction 2 were 62.5% and 60% for 100 IU/kg and 150 IU/kg dose levels, respectively, Table 2.7.3-34.

Relapse, EFS and OS

Fourteen patients (46.7%) suffered from a relapse (reported during the whole study), with a higher proportion in the 100 IU/kg group (7 patients, 53.8%) than in the 150 IU/kg group (6 patients, 42.9%). Relapses were mainly medullary (13 out of 14).

At the time of the analysis at 24 months, EFS rate was slightly higher in the 150 IU/kg group (N=12, 85.7%) than in the 50 IU/kg group (N=2, 66.7%) and 100 IU/kg group (N=10, 76.9%). There was no statistically significant improvement in EFS between treatment groups (Log rank p-values > 0.05)

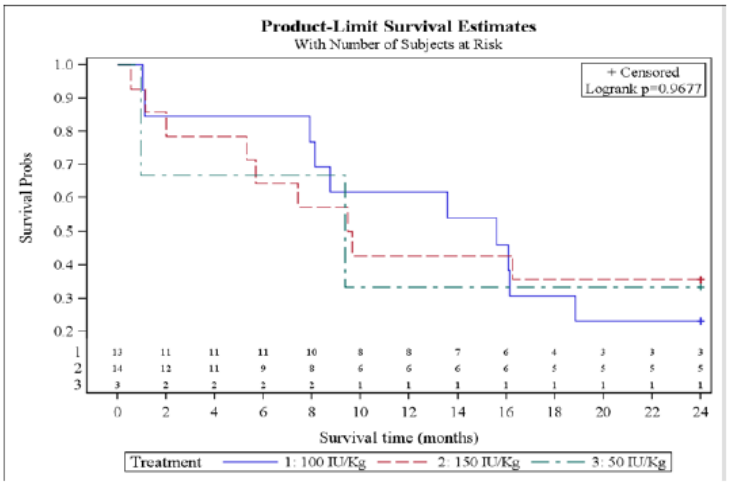
At the time of survival analysis at 24 months, 21 patients (70.0%) were dead; 2 (66.7%) patients treated with 50 IU/kg, 10 (76.9%) patients treated with 100 IU/kg, and 9 (64.3%) patients treated with 150 IU/kg. The log rank tests with treatment as the only factor did not demonstrate any statistically significant difference in OS between treatment groups, Figure 2.7.3- 9.

Table 2.7.3- 34 Summary of CR and MRD rates - Study GRASPALL/GRALL SA2-2008

		Treatment dose (Number (%) of patients)			
		50 IU/kg N=3 (%)	100 IU/kg N=13 (%)	150 IU/kg N=14 (%)	Total N=30 (%)
CR rate					
Induction 1	N	2	13	13	28
	Yes	2 (100.0)	10 (76.9)	8 (61.5)	20 (71.4)
	No	0	3 (23.1)	5 (38.5)	8 (28.6)
	Missing values	1	0	1	2
Induction 2	N	2	11	9	22
	Yes	2 (100.0)	10 (90.9)	7 (77.8)	19 (86.4)
	No	0	1 (9.1)	2 (22.2)	3 (13.6)
	Missing values	1	2	5	8
MRD rate					
End of Induction 1					
	N	2	9	5	16
	Negative (< 10^{-3})	0	5 (55.6)	2 (40.0)	7 (43.8)
	Positive (≥ 10^{-3})	2 (100.0)	4 (44.4)	3 (60.0)	9 (56.2)
End of Induction 2					
	N	2	8	5	15
	Negative (< 10^{-3})	2 (100.0)	5 (62.5)	3 (60.0)	10 (66.7)
	Positive (≥ 10^{-3})	0	3 (37.5)	2 (40.0)	5 (33.3)

Source: Table 19 and Table 20 of the CS

Figure 2.7.3- 8 Plot of Kaplan-Meier comparison of OS at 24 months- Study GRASPALL/GRALL SA2-2008



PK/PD parameters

Asparaginase concentration over time

Plasma: The mean plasma Asp concentration was 2.9 ± 4.4 µmol/L before injection, then increased until D10 (15.2 ± 5.6 µmol/L), and finally decreased until the end of induction (8.3 ± 7.6 µmol/L). Similar trend was observed during induction 2.

A few days after administration, free asparaginase values were low (under 1 U/L): indeed, free asparaginase values were 0.52 U/L and 0.48 U/L at respectively D10 of induction 1 and D13 of induction 2, i.e. 7 days after each injection.

Encapsulated asparaginase: decreased gradually after Graspa administration during induction 1 and induction 2. Indeed, the following mean encapsulated asparaginase values were reported chronologically at each timepoint: 1496 U/L after first injection then 1148 U/L, 752 U/L and 583 U/L at the end of induction 1 and 1450 U/L after second injection then 1219 U/L, 910 U/L and 724 IU/L at the end of induction 2.

GraspaNC 2008-02 Trial

This study has been conducted in a completely different target population and is just briefly presented for information

Title:Phase I, dose escalation clinical trial of Graspa (Red Blood Cells encapsulating L-Asparaginase) in patients with pancreatic carcinoma

Objectives

- To determine Maximal Tolerated Dose of Graspa for patients with locally advanced or metastatic pancreatic adenocarcinoma whose first or second chemotherapy with gemcitabine has failed.
- To evaluate tumour response based on progression of tumour volume
- To evaluate the pharmacodynamic and pharmacokinetic parameters of Graspa

Methodology

Dose escalation study with 4 doses: 25, 50, 100 and 150 IU/Kg, open label. Each dose was administered once to cohorts of 3 patients. The second patient of each dose group was included after 4 weeks of safety monitoring of the first patient. If no patient has a limiting toxicity, the next dose level was tested with 3 new patients. If 1 out of 3 patients had a limiting toxicity, the same dose level was tested with three new patients. If at least two patients out of 3 displayed a limiting toxicity, inclusion should be stopped. The MTD would be the previous lower dose.

Patients

12 patients were included and analysed corresponding to 1 cohort of 3 patients per dose.

Inclusion criteria: exocrine pancreatic adenocarcinoma cytologically or histologically confirmed, non-resectable locally advanced or metastatic (stage IV) as defined by TNM 2002 classification (UICC 2002) and resistant to a first or second line chemotherapy including gemcitabine; between 18 to 70 years

Exclusion criteria: endocrine or acinar pancreatic tumour or with known or suspected cerebromeningeal metastases;Splenic vein thrombosis < 3 months or under active treatment;Haemoglobin level greater than 13 g/L;Additional criteria in line with those in GRAALL 2008 study

Endpoints

Primary criteria:Evaluation of MTD 1 month following injection. Evidence of dose limiting toxicities:

- grade 2,3 or 4 for pancreatic toxicities, and /or
- grade 3 or 4 for allergies (hypersensitivity), neurological, hepatic and /or
- coagulation toxicities
- all other grade 4 toxicities

Results

MTD

No patients experienced dose-limiting toxicities between baseline and D28. Consequently, according to the protocol, the MTD is 150 IU/Kg. No patients experienced dose-limiting toxicities as well between D28 and end of study.

Safety

During the study, ten patients were withdrawn: One patient died during the study, 8 dropped out for progressive disease and one for informed consent withdrawal.

3 SAEs occurred during the trial, not related to the product according investigators assessment. 6 patients experienced 12 adverse events out of 66 reported related to the study drug:

Efficacy

Mean duration of asparagine depletion was 6.44 ± 3.44, 5.57 ± 4.40, 4.76 ± 4.69, and 12.89 ± 6.88 days in the 25, 50, 100 and 150 IU/Kg respectively.

No signal was observed with regards to biological tumour response or tumour volume.

The mean plasma Glutamine and glutamic acid concentrations had the same profile and remained steady over time whatever the dose, suggesting that Graspa did not present glutamine activity as could be expected with a free L-asparaginase

Half-lives were respectively 20.21 days, 18.87 days, 17.87 days and 26.76 days for 25, 50, 100 and 150 IU/kg groups.

No safety issue was observed in this study up to the maximum dose of Graspa administrated. Therefore, the DMT has been set at 150 IU/Kg. This dose will be used for subsequent clinical development.

3.3.6 Discussion on clinical efficacy

The initially claimed indication for Graspa read as follows: “Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of paediatric and adult patients with acute lymphoblastic leukaemia, who have either relapsed or failed first line treatment. Graspa is also indicated for the treatment of paediatric and adult patients with acute lymphoblastic leukaemia and hypersensitivity to asparaginase.” This includes a broad indication regardless of the line of treatment in patients with HS to asparaginase and a second line treatment for the general population. It also includes induction, consolidation and maintenance and any multi-agent chemotherapeutic regimen.

Following criticisms to the evidence presented in support of the efficacy in the subset of patients with prior HS to asparaginase, a revised indication is now provided:

“Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of children over 1 year of age and adult patients with Philadelphia chromosome negative acute lymphoblastic leukaemia, who have either relapsed or failed first line treatment. “

The efficacy co primary endpoint for the pivotal study was duration of asparaginase activity >100U/L. This was seen to be significantly longer in the Graspa non-allergic group than in the L-asparaginase comparator arm. The mean duration (95% confidence interval) difference was 11.02 (7.5; 14.6) days; p <0.001. The differences in mean duration at each stage of the trials (interim 1, between interim 1 an interim 2, and after interim 2) were: 14.45 (10.07; 18.83), 10.25 (3.95; 16.54), and 8.58 (-2 01; 19.17) days, respectively (calculation using the Fisher combination test resulted in a p-value < 0.001). Extreme levels of significance were also obtained using the bootstrap method at all stages of the trial. Both analyses confirm the superiority of Graspa compared to L-ASP with regard to this component of the co-primary endpoint. The results of the sensitivity analyses, which considered duration of asparaginase activity above 75 and 400U/L were similar to those of the primary analysis; as were the subgroup analyses, which showed similar differences between Graspa and L-asparaginase in the subgroups of children, induction regimen, antibody status and risk score. For adults, the mean duration in the L-asparaginase arm was very low (2.3 days) and may reflect the low numbers of patients in the

subgroup or early termination of L-asparaginase treatment due to adverse events. In all patients for both Graspa and L-asparaginase arms, duration of activity was shorter in those positive for anti-drug antibodies at baseline. This phenomenon was also seen in the secondary measures of efficacy likely related to the reduced exposure to asparaginase. In the asparaginase allergic patients the duration was slightly shorter than for the non-allergic group; yet still longer compared to the L-asparaginase group.

With regard to the safety co-primary end point, there were no allergic reactions related to study treatment in the Graspa group. The difference in proportions (95% confidence interval) was 46% (24%, 69%); $p < 0.001$. The results are consistent for patients included before the first interim analysis and those recruited after the first interim analysis and before the second interim analysis. Results from the subgroup analyses are generally consistent with the primary analysis. As is seen for most asparaginases, most ADA positive patients had HS reactions during induction.

It could therefore be concluded that the aim of the study had been met and efficacy had been shown. However, there are a number of issues which call the use of 'mean duration of asparaginase activity>100U/L in whole blood' as a surrogate measure of asparaginase efficacy in this setting into question. These include:

- Choice of control treatment regimen

  1 - the lower than SmPC recommended daily dose for L-asparaginase/ higher doses used in clinical practice

  2 - the shorter period of L-asparaginase administration (especially for VANDA induction)

  3 – the presence of an interval between F1 and F2 L-asparaginase dosing

  4 – the use of a non-PEGylated asparaginase as a comparator, given the patient population (relapsed disease, known hypersensitivity to native asparaginase), the widespread use of PEGylated asparaginase to treat ALL in clinical practice and the prolonged exposure of the product, which resembles that of Graspa.

- Evaluation of asparaginase activity in whole blood

  The results of the asparaginase activity evaluation asexpected do not reflect the activity of the product in its biologically active form (compartmentalised within erythrocytes) as erythrocyte lysis, releasing free asparaginase, is required to assay asparaginase in whole blood. Whilst the whole blood asparaginase activity peaks at 2133U/L, far higher than is required to achieve complete asparagine depletion instantaneously and much higher than the activity levels seen with the comparator, time to asparagine depletion was longer than that of the comparator and duration and incidence of asparagine depletion were notably shorter and smaller respectively. The PK data for Graspa are not compatible with the PD outcomes. This evidence suggests that the asparaginase activity of Graspa as measured in whole blood, vastly overstates the activity of the product in vivo.

- Threshold activity of >100U/L

  Historically, this may have been the accepted threshold to ensure asparagine depletion in both serum and CSF in the majority of patients. However, more recently published and proprietary data from asparaginase manufacturers have shown that activity levels as low as 20 U/L are still therapeutic and may still result in complete asparagine

depletion in both fluids. Given these more recent findings, analyses of duration of asparaginase activity, using lower thresholds e.g. 20U/L and 50U/L, should have at least been provided.

Statistically significantly higher rates of cytologic and complete remission at the end of induction were seen in the Graspa arm, with results showing some consistency in effect. Whilst not statistically significant, similar trends were seen in the subgroups. Overall complete and cytological remission were more frequent in children and those with no ADAs at baseline. This is expected and is in line with known prognostic factors. However, there are some issues with the evaluation of complete remission Evaluation of CR should have included CSF evaluation and objective evaluation of extramedullary disease. Further the CR endpoint definition should have incorporated all features of both clinical and cytologic remission. It was also unclear whether those who received rescue treatments before the end of induction were included in the analyses.

Overall survival and event free survival analyses also showed a trend to better efficacy with Graspa, although only 12-month follow up data were available and overall the data could not be considered mature. Whilst there are trends to differences between Graspa and L-asparaginase in the OS and EFS data, the data for relapse rate do not appear to show differences between the study arms and overall are low. There is a suggestion therefore that patients may be dying from causes other than relapse/ disease progression and that the differences in OS and EFS between the study arms may not be related to differences in efficacy between the two trial products. MRD rates are similar between the 'non-allergic' study arms. Reasons for the trend to better CR, OS and EFS data for Graspa are unclear, given its significantly weaker effect on depleting plasma asparagine compared to L-asparaginase. The differences in mean age and proportion of patients in the younger age group(1-11 years) (which has better prognosis) between the study arms should be considered here.

There were clear clinically relevant differences in mean duration of plasma asparagine depletion and proportion of patients with at least one day of asparagine depletion between the two asparaginase non-allergic study arms (L-asparaginase > Graspa). These data showing a significantly greater effect of L-asparaginase than Graspa on asparagine clearance were in direct opposition to the other measures of efficacy in the development programme.

As expected the mean duration of depletion was shorter in patients with previous allergy to L-asparaginase than in those without prior allergy, as patients with prior allergy have a higher incidence of anti-asparaginase antibodies and/ or higher clearance of asparaginase through other mechanisms. What is striking about the asparagine depletion data is the extent of the differences between study arms despite the identified bias within the study design against the L-asparaginase arm and Graspa activity estimates being >10 x the activity levels thought to ensure complete asparagine depletion in plasma and CSF and that its activity readouts throughout the study were much higher than asparaginase activity values in the comparator arm.

It is clear that despite its activity in whole blood, Graspa's ability to hydrolyse plasma asparagine is significantly impaired as almost all the asparaginase is sequestered within erythrocytes and its activity is limited by the transport of asparagine across the erythrocyte membrane and less importantly lysis of erythrocytes. With regard to establishing the anti-leukaemic effect of asparaginases the critical factor is the capacity to deplete asparagine from the plasma, as this is the main source of asparagine for leukaemic cells, as it cannot be synthesized intracellularly. Therefore, despite the presence of asparaginase in large quantities in whole blood, Graspa may not have a profound anti-leukaemic effect, due to the above limitations in hydrolysing asparagine.

Further to the discussion above, the CHMP currently consider duration of asparagine depletion to be the most appropriate and clinically relevant surrogate measure for efficacy of asparaginases. Against what is argued by the Applicant, ex-vivo depletion of asparagine in the L-asparagine assay is not considered to be a significant factor and not thought to unduly bias against Graspa the estimation of plasma asparagine. Sampling for asparagine in both study arms in study GraspaLL 2009-06 occurred at baseline, followed by a few days of daily sampling and then every third day, just prior to administration of native asparaginase. Available ex-vivo data from recent asparaginase MA applications show the superlative kinetics of asparaginase. Taking the enzyme's kinetics into consideration, it is not clear how ex-vivo depletion of asparagine is considered important, given that at the point of sampling for asparagine levels at most points in the study, asparaginase has already been active in the patient's blood stream for 24-72 hours. It is not clear how much of a difference a few additional seconds/minutes of blood exposure to asparaginase (as samples are processed) would make. Even when in-vivo replenishment of asparagine, through endogenous and exogenous processes, are taken into account, the contribution of ex-vivo asparagine hydrolysis to overall asparagine depletion is expected to be small and its impact on the comparison of asparaginase depletion between L-asparaginase and Graspa (where it is accepted ex-vivo depletion is unlikely to occur), negligible. The Applicant has been asked to clarify and to support its assertion that in this development programme incidence and duration of asparagine depletion cannot be used as the primary surrogate measures of efficacy.

Three MO were raised at D120 in order to address the previous concerns.

Regarding the doubts around the control treatment regimen, the Applicant argued that the choice of asparaginase formulation in this setting is largely driven by local practice and nationally accepted standard of care protocols. The native L-ASP was used in the pivotal trial, since Pegylated-asparaginase was not approved at the time in France. So, it is acknowledged that the comparison to an unauthorised treatment would have been difficult, even if it was already commonly used in clinical practice as shown by the switches in the Graspa arm, which rarely involved native L-ASP. In addition, it is acknowledged that the selected dose is the highest recommended dose and the frequency of 3 administrations per week is common practice (although this is not that recommended in the French SmPC, which mentions for re-induction daily injections for 5 to 15 days). During the induction phase, there is no justification for the use of a longer interval (5 days) between the two courses of asparginase (4 injections) combined with the F blocks. Asparaginase plasma concentrations were low (median 6.6 U/ml) before the beginning of the second course. When combined with VANDA, in high risk patients, the number of injections was only 4, **and not 8 as stated by the Applicant**, as there was only one treatment block. As this was the standard protocol used by the European Organization for Research and Treatment of Cancer - French Acute Lymphoblastic Leukaemia group, it is recognised that it may have been difficult to impose another protocol for this study. Therefore, the administration regimen of L-ASP is not considered optimal but it is acceptable as it is used in clinical practice.

In conclusion, although the COOPRALL protocol is not considered optimal, the comparison could in principle be acceptable. However, further information is requested before considering this issue solved. The Applicant should describe a range of other protocols used in clinical practice and discuss how the results of the pivotal study can be reasonably generalised to these other protocols. Regarding the internal validity of the pivotal trial, the Applicant should specify the proportions of patients who received L-ASP via the IV and IM routes. The Applicant should provide and comment on the missing table about treatment compliance.

MO was raised at D120 in order to try to better understand the validity of the proposed pharmacodynamic endpoint as valid surrogates of efficacy, in order to draw conclusions on the non-inferiority of Graspa vs native L-asparaginase, both in combination with standard chemotherapeutic

regimens for the treatment of ALL in 2nd line. Experimental tests provided indicate that ex-vivo degradation might exist as long as sufficient asparaginase activity at plasma level exists, thus measurements of asparagine depletion in plasma might be underestimated. Data presented show that asparaginase activity in plasma remains at effective depleting levels across the entire induction period sampling in the L-asp native arm whilst for Graspa, asparaginase activity in plasma is below the 100 IU/L cut off in all samples. Therefore, a differential effect on ex vivo asparagine depletion cannot be firmly ruled out. The main problem being that the magnitude of the ex-vivo degradation in each treatment arm cannot be estimated reliably and thus, it can hardly be relied on asparagine depletion measurements for the comparison between treatment arms. So, at present it is uncertain to what extent differences in mean duration of asparagine depletion are due to ex-vivo degradation and/or to real differences in activity between treatment arms, making difficult drawing conclusions from these data.

However, with Graspa, the ASN plasma levels are reasonably accurate given the low level of asparaginase activity in plasma, and the conclusion that ASN depletion is often lacking with Graspa remains correct based on current definitions of ASN depletion. The difficulty is that the levels of depletion that have been correlated in the past with successful efficacy outcomes for classical asparaginase products have been underestimated. Therefore, it can be agreed with the Applicant that it is likely that higher concentration of ASN than those currently targeted may still be clinically effective but it is currently not known what these levels are. Nevertheless, this could explain why clinical efficacy outcomes (CR, survival) do not appear worse than with L-ASP.

Data presented show that the levels of asparaginase in plasma following Graspa administration are marginal and thus, asparaginase activity is mainly due to the encapsulated one. The results still indicate long-lasting whole blood duration of asparaginase activity over 100 IU/l in Graspa, longer than for L-asp, but inconsistent with the limited duration of asparagine depletion. So, asparaginase activity might be overestimated in the Graspa treated arm since despite the presence of so called “active levels” (in whole blood), these are unable to deplete asparagine as expected. Therefore, requirements for asparaginase activity as defined for classical asparaginase products cannot be used in the case of Graspa.

In summary, asparagine depletion results might bias comparison in favor of L-asp, in an unknown magnitude, whilst asparaginase activity (in whole blood for Graspa vs plasma for L-asp) appears to overestimate the treatment effect in favor of Graspa in a substantial amount. Therefore, no reliable comparisons can be made based on PD markers. Since PD markers are not helpful at present to adequately predict the efficacy of Graspa, this application mainly relies on clinical outcomes. Due to the limited sample size of the pivotal trial, the estimates of efficacy are notably uncertain and non-inferiority of Graspa vs L-ASP cannot be established. This remains currently a major concern.

If Graspa is authorised, there is a need for an appropriate target ASN concentration so that treatment is not unduly stopped. The Applicant should provide the lower ASN concentration achieved during the induction phase before the date of CR assessment (individual listing in both allergic and non-allergic patients) and the distribution of this parameter in those patients who achieved CR vs those who did not. Although based on limited data, this could suggest an ASN level that is sufficient to produce a favourable outcome and might be used to monitor Graspa treatment in a future trial.

Overall survival data are not considered sufficiently sensitive to detect a difference between study drugs and can only be used for insurance that no obvious harm emerges. Furthermore, this analysis is confounded but the other asparaginase products received.

Immunogenicity of Graspa is another key information that is currently missing since the Applicant has admitted that the ADA assays have not been validated.

Finally, hypersensitivity to asparaginase, usually with development of neutralising antibodies, has proved an important adverse reaction with approximately 5% of patients being allergic to Erwinase, the least allergenic product. Pegaspargase is also less immunogenic and the incidence of hypersensitivity tends to be lower. However, in patients with prior exposure to native asparaginase E.

coli, the incidence has been reported as high as 50% for pegaspargase. Erwinase is licensed for use in patients with previous hypersensitivity reactions, whilst pegaspargase is frequently used in those with previous hypersensitivity to native L-asparaginase. One of the requested indications for Graspa is for use in those with hypersensitivity to asparaginase. However, no comparison was made with pegaspargase or Erwinase. Further, it is noted that patients with grade 4 hypersensitivity reactions were excluded. It is therefore not clear whether treatment with these agents would be more successful that treatment with Graspa and whether treatment with Graspa is less allergenic or whether it can be used successfully in those who have reacted to Erwinase. It is not clear where in the line of different asparaginases this product would be recommended. As such the evidence supporting the proposed indication for use in patients with prior hypersensitivity to L-asparaginases is not considered compelling. In the responses to D120, the Applicant clarifies that this part of the indication is not further pursued and present a revised wording which excludes any reference to the subset of patients with HS to asparaginase. So, there is no need for further discussing on this issue.

3.3.7. Conclusion on clinical efficacy

The major concerns related to the demonstration of efficacy of this asparaginase formulation remain unsolved. The validity of the comparison based on PD parameters, either asparagine depletion or asparaginase activity, is seriously questioned and thus the non-inferiority in the activity of Graspa vs native L-asparaginase cannot be established. In the absence of reliable PD parameters, further discussion and analysis are needed to conclude on the validity of the conventional clinical endpoints from the pivotal study in order to demonstrate the non-inferiority in efficacy of Graspa in comparison to native L-asparaginase.

3.3.8. Clinical safety

The safety profile of Graspa as combination therapy with other treatments has been evaluated in a total of 189 patients with ALL, AML or solid tumours in 8 completed or ongoing ERYTECH sponsored clinical studies in Europe and US.

At the time of the data cut-off, three clinical studies with Graspa were completed in children and/or adults with ALL in combination with multi-agent chemotherapy. A total of 189 patients received Graspa in the clinical programme (ongoing and completed studies) as of the clinical cut-off date of 2nd March 2015. In clinical studies for which final clinical study reports (CSRs) are included in this submission, a total of 114 patients with cancer received Graspa.

Table 2.7.4- 1 Tabulation of patients in completed and ongoing studies with GRASPA- Safety Population

Study population	Combination			Monotherapy
Completed studies (final CSR included in the submission)				
	ALL	**AML**	**Pancreatic**	**Pancreatic**
GRASPALL 2009-06	54[a]			
GRASPALL 2005-01	18			
GRASPALL GRAALL SA2 2008	30			
GRASPANC 2008-02				12
Ongoing studies				
GRASPA-AML 2012-01 (Appendix 6)		49		
GRASPALL 2012-10 EAP [b] (Appendix 8)	12			
GRASPALL 2012-09 (Appendix 7)	3			
GRASPANC 2013-03			11	
Total	**117**	**49**	**11**	**12**

a : 52 patients randomized and received GRASPA, and 2 patients switched treatment from L-ASP to GRASPA
b : EAP ; Expanded Access Program
Data Source: Overview of Pivotal and supportive studies (Appendix 1)

Patient exposure

Table 2.7.4- 5 Number (%) of patients exposed to GRASPA – Safety population

	GRASPA 25 IU/Kg N (%)[1]	GRASPA 50 IU/Kg N (%)[1]	GRASPA 100 IU/Kg N (%)[1]	GRASPA 150 IU/Kg N (%)[1]	Total N (%)
Completed studies in ALL					
GRASPALL 2005-01	0	6 (66.7)	6 (31.6)	6 (8.1)	18 (17.6)
GRASPALL 2009-06	0	0	0	54 (73.0)	54 (52.9)
GRASPALL/GRAALL	0	3 (33.3)	13 (68.4)	14 (18.9)	30 (29.4)
Subtotal	**0**	**9**	**19**	**74**	**102**
Other studies in Global Program *					
GRASPALL 2012-09	0	3 (20.0)	0	0	3 (1.6)
GRASPA-AML 2012-01	0	0	49 (59.8)	0	49 (25.9)
GRASPANC 2008-02	3 (100.0)	3 (20.0)	3 (3.7)	3 (3.4)	12 (6.3)
GRASPANC 2013-03	0	0	11 (13.4)	0	11 (5.8)
GRASPALL2012-10-EAP	0	0	0	12 (13.5)	12 (6.3)
Total in global program	**3**	**15**	**82**	**89**	**189**

N corresponds to the number of patients in each treatment group
[1] % corresponds to the percentage of patients in each dose level and pooled population.
*Studies are all on going except Graspanc 2008 completed in 2012
Source: Table 1.3 of Global safety output (Appendix 9)

Table 2.7.4- 6 Number (%) of patients exposed to GRASPA – ALL Safety population

		GRASPA				L-ASP
Study	**Subgroup**	**50**	**100**	**150 [a]**	**TOTAL**	
2009-06	N			54 [b]	54	28
	Allergic adults			11	11	NA
	Allergic children			15	15	NA
	Non-allergic adults			5	5	7
	Non-allergic children			21	21	21
2005-01	N	6	6	6	18	6
	Adults	3	3	3	9	3
	Children	3	3	3	9	3
SA2-2008	Adults >55	3	13	14	30	NA
All Studies	N	9	19	74	102	34
	Children	3	3	41 [b]	47 [b]	24
	Adults	6	16	33	55	10

N corresponds to number of patients in each treatment group
[a] not all patients received the intended full dose of GRASPA
[b] includes 2 patients who switched from L-ASP to GRASPA
Source: CSR 2005-01, CSR SA2-2008, CSR 2009-06.

Adverse events

In the entire programme, 93.1% of patients experienced at least one AE, of which 70.9% of the patients had events that were considered related to the study drug. The majority of the patients (77.2%) experienced at least one SAE; however, only 24.9% had related SAEs, with 4.8% had related SAEs with a fatal outcome. The reported AEs are not unexpected for this population.

In the 3 key studies (pivotal Phase II/III and supportive studies), all patients experienced at least one AE; of these 80.4% had related AE. The majority of the events resolved, with 15.7% of the patients reported AEs leading to either temporary or permanent discontinuation of the study drug. The majority of the patients (89.2%) experienced at least one SAE, with 27.5% reporting SAEs that were considered study-drug related. The related SAEs with a fatal outcome accounted for 3.9%.

In safety population of completed ALL studies, AE rate was similar in children and adults. However, adult population (N= 55) had a higher rate of events leading to study drug discontinuation (23.6% vs. 6.4%), related SAEs (41.8% vs. 10.6%), SAEs with fatal outcome (65.5% vs. 17.0%), and related SAEs with fatal outcome (7.3% vs. 0%). This pattern of higher rates in adults reflects the presence of comorbidities in this population, and is not unexpected.

Impaired coagulation, which is a known class effect with L-asparaginase therapy and was reported with all other L-asparaginase formulations, was the most common AE (52.9%) in the safety population of the completed ALL studies, as well as in the entire safety population (second to thrombocytopenia), accounting for 39.2%. It was serious in 2 patients. Grade 3 and Grade 4 impaired coagulation accounted for 16.9% of the patients.

Hepatic and pancreatic toxicities are AEs uniformly associated with L-asparaginase therapy. The overall incidence of elevated transaminases and hepatic toxicity were 25.9% and 25.4%, respectively.

The incidence of these events considered related to the study drug was 14.8% and 13.8%, respectively. Hyperbilirubinaemia accounted for 13.8%, and most of these events were considered related to the study drug (10.0%). Asymptomatic elevation of amylases and lipases (biochemical pancreatitis) occurred in 39 (20.6%) patients, in which most of these events were study drug-related (16.4%). Of the 39 patients, 6 (3.2%) patients had clinical manifestations of pancreatitis.

Many causes of hyperglycaemia exist in patients with ALL, and frequently the reason is multifactorial, which could be attributed to the disease characteristics, the heavy use of corticosteroids, and/or to L-asparaginase therapy. In the completed ALL studies, hyperglycaemia occurred in 26 (25.5%) patients. These AEs were related in 7.4% of the patients.

No patient experienced neurotoxicity, or osteonecrosis in the Graspa programme. In addition, myelosuppression, including thrombocytopaenia, neutropenia, lymphopaenia and pancytopaenia are frequent events in patients with ALL and AML, reflecting the disease characteristics and the effect of the multi-agent chemotherapy.

The safety population included patients treated at 3 Graspa dose levels: 50 IU/Kg, 100 IU/Kg, and 150 IU/Kg. The pivotal study GraspaLL 2009-06 had only the 150 IU/Kg dose. The other 2 supportive studies (Study GraspaLL 2005-01 and Study GraspaLL GRAALL SA2 2008) were dose escalation studies, and therefore had these 3 dose levels. In addition, the AML study is evaluating Graspa at 100 IU/Kg dose level. Overall, the incidence of AEs is lower at the 50 IU/Kg dose level. At the higher dose levels, there are no notable differences, although there is a trend towards higher proportion of AEs with 150 IU/Kg dose level compared to the 100 IU/Kg dose level.

Table 2.7.4- 14 Number (%) of patients with ≥ 10% AE by Preferred Term and GRASPA dose levels – Safety population

Preferred Term [a]	Global Program population		
	50 IU/kg N = 15 (%)	100 IU/kg N = 82 (%)	150 IU/kg N = 89 (%)
At least one adverse event	15 (100.0)	76 (92.7)	82 (92.1)
Thrombocytopenia *	1 (6.7)	35 (42.7)	49 (55.1)
Impaired coagulation parameter *	4 (26.7)	27 (32.9)	43 (48.3)
Neutropenia	0	13 (15.9)	47 (52.8)
Febrile bone marrow aplasia	5 (33.3)	12 (14.6)	38 (42.7)
Leukopenia	0	6 (7.3)	48 (53.9)
Transaminases increased *	5 (33.3)	8 (9.8)	36 (40.4)
Hepatotoxicity *	3 (20.0)	15 (18.3)	30 (33.7)
Drug hypersensitivity *	3 (20.0)	14 (17.1)	30 (33.7)
Anemia *	0	28 (34.1)	16 (18.0)
Sepsis *	3 (20.0)	18 (22.0)	23 (25.8)
Biochemical pancreatitis *	1 (6.7)	14 (17.1)	24 (27.0)
Mucosal inflammation	2 (13.3)	6 (7.3)	31 (34.8)
Pyrexia *	1 (6.7)	18 (22.0)	13 (14.6)
Hyperglycemia *	5 (33.3)	11 (13.4)	15 (16.9)
Asthenia *	3 (20.0)	20 (24.4)	5 (5.6)
Leukemia recurrent	1 (6.7)	6 (7.3)	21 (23.6)
Lymphopenia *	1 (6.7)	3 (3.7)	23 (25.8)
Gastrointestinal motility disorder *	2 (13.3)	15 (18.3)	7 (7.9)
Hyperbilirubinemia *	2 (13.3)	13 (15.9)	11 (12.4)
Blood albumin decreased	2 (13.3)	3 (3.7)	19 (21.3)
Nausea	2 (13.3)	17 (20.7)	3 (3.4)
Hypokalemia *	0	7 (8.5)	16 (18.0)
Febrile neutropenia	0	7 (8.5)	15 (16.9)
Hyponatremia *	1 (6.7)	7 (8.5)	14 (15.7)
Other causes of hypersensitivity *	2 (13.3)	14 (17.1)	5 (5.6)
Vomiting	1 (6.7)	11 (13.4)	6 (6.7)

N corresponds to the number of patients in each treatment group
[a] For each preferred term patients are included only once even if they experienced multiple events in that preferred term.
*Pooled Preferred Term as defined in the Safety Analysis Plan
Source : Table 2-15 of the Global safety output (Appendix 9).

Study GraspaLL 2009-06

The majority of events occurred at a lower frequency with Graspa compared to L-ASP. Thus, impaired coagulation disorders included hypofibrinogenaemia (30.8% vs. 67.9%), decreased anti-thrombin III (15.4% vs. 71.4%), prolonged activated partial thromboplastin time (PTT) (3.8% vs. 14.3%), and decreased prothrombin level (0% vs. 10.7%), in the Graspa and L-ASP arms, respectively.

There was a lower incidence of hypersensitivity reactions (all events attributed to L-asparaginase or non-asparaginase treatments), in Graspa than in L-ASP arm, accounting for 19.2% and 64.3% of patients, respectively. Elevated AST and/or ALT occurred in 57.7% and 42.9% of patients in the Graspa and L-ASP arms, respectively. Hyperbilirubinaemia and hepatotoxicity accounted for 7.7% and 11.5% in the Graspa arm, compared to 28.6% and 21.4% in L-ASP arm. A total of 34.6% and 57.1% had elevation of pancreatic enzymes in Graspa and L-ASP arms, respectively. A similar pattern was observed with hyperglycaemia (15.4% and 28.6%) and hypertriglyceridemia (7.7% and 10.7%).

In the Graspa-s arm (allergic patients), drug hypersensitivity reactions occurred in 57.7%. AEs related to coagulation disorders were reported at varied frequencies ranging from 0% (decreased prothrombin level) to 34.6% (decreased anti-thrombin III). Other AEs of interest included: elevated transaminases

(46.2%); hypofibrinogenaemia (38.5%); GGT increased (30.8%); hepatotoxicity (30.8%); hypoalbuminaemia (19.2%); and hyperbilirubinaemia (19.2%). Lipase and/or amylase enzymes were also elevated in 38.5% of patients.

Table 2.7.4- 15 Summary of most frequently reported AEs in ≥ 10% of non-allergic patients arranged by MedDRA Preferred Term - Study GRASPALL 2009-06

Preferred Term [a]	GRASPA N=26 (%)	L-ASP N= 28 (%)
Leukopenia *	23 (88.5)	25 (89.3)
Thrombocytopenia *	21 (80.8)	26 (92.9)
Neutropenia	20 (76.9)	23 (82.1)
Mucosal inflammation	18 (69.2)	12 (42.9)
Febrile bone marrow aplasia	15 (57.7)	15 (53.6)
Transaminases (ALT and/or AST) increased*	15 (57.7)	12 (42.9)
Lymphopenia	11 (42.3)	13 (46.4)
Hypokalemia *	10 (38.5)	8 (28.6)
Biochemical pancreatitis (amylase and/or lipase elevation)*	9 (34.6)	16 (57.1)
Hypofibrinogenemia	8 (30.8)	19 (67.9)
Anemia	7 (26.9)	8 (28.6)
Gamma-glutamyltransferase (GGT) increased	6 (23.1)	10 (35.7)
Sepsis	6 (23.1)	3 (10.7)
Drug hypersensitivity reactions	5 (19.2)	18 (64.3)
Hypoalbuminemia	5 (19.2)	11 (39.3)
Anti-thrombin III decreased	4 (15.4)	20 (71.4)
Other allergic reactions*	4 (15.4)	5 (17.9)
Febrile neutropenia	4 (15.4)	8 (28.6)
Hyperglycemia/Diabetes mellitus *	4 (15.4)	8 (28.6)
Hyponatremia *	4 (15.4)	8 (28.6)
Vomiting	4 (15.4)	2 (7.1)
Diarrhea	3 (11.5)	3 (10.7)
Graft versus host disease	3 (11.5)	2 (7.1)
Recurrent leukemia	3 (11.5)	4 (14.3)
Pyrexia	3 (11.5)	3 (10.7)
Septic shock	3 (11.5)	1 (3.6)
Staphylococcal infection	3 (11.5)	1 (3.6)
Staphylococcal sepsis	3 (11.5)	1 (3.6)
Streptococcal infection	3 (11.5)	0
Hyperbilirubinemia	2 (7.7)	8 (28.6)
Hepatotoxicity *	3 (11.5)	6 (21.4)
Pancreatitis*	2 (7.7)	4 (14.3)
Hypertriglyceridemia *	2 (7.7)	3 (10.7)
Decreased appetite	2 (7.7)	3 (10.7)
Abdominal pain	1 (3.8)	4 (14.3)
Activated partial thromboplastin time (PTT) prolonged	1 (3.8)	4 (14.3)
Lung disorder	1 (3.8)	3 (10.7)
Prothrombin level decreased	0	3 (10.7)
Pain	0	3 (10.7)

N corresponds to the number of patients in treatment groups
[a] For each Preferred Term (PT) patients are included only once even if they experienced multiple events in that PT.
*Pooled Preferred Term as defined in the Statistical Analysis Plan.
Source: Table: 9-3 of CSR

Table 9-4 Summary of most frequently reported AEs in ≥ 10% of patients in ERY001-S Arm, arranged by MedDRA Preferred Term - Safety population

Preferred Term [a]	ERY001-s N=26 (%)	
	Induction	Overall study [b]
Thrombocytopenia *	24 (92.3)	25 (96.2)
Neutropenia	20 (76.9)	25 (96.2)
Leukopenia *	23 (88.5)	24 (92.3)
Drug hypersensitivity reactions*	9 (34.6)	15 (57.7)
Febrile bone marrow aplasia	9 (34.6)	14 (53.8)
Transaminases increased*	8 (30.8)	12 (46.2)
Lymphopenia	8 (30.8)	11 (42.3)
Biochemical pancreatitis (amylase and/or lipase)*	8 (30.8)	10 (38.5)
Hypofibrinogenemia	9 (34.6)	10 (38.5)
Mucosal inflammation	5 (19.2)	9 (34.6)
Anti-thrombin III decreased	6 (23.1)	9 (34.6)
GGT increased	6 (23.1)	8 (30.8)
Other allergic reactions*	1 (3.8)	4 (15.4)
Anemia	6 (23.1)	7 (26.9)
Febrile neutropenia	5 (19.2)	7 (26.9)
Hepatotoxicity *	6 (23.1)	8 (30.8)
Hyponatremia *	4 (15.4)	6 (23.1)
Pyrexia	1 (3.8)	6 (23.1)
Hypoalbuminemia	5 (19.2)	5 (19.2)
Hyperglycemia/Diabetes mellitus *	4 (15.4)	5 (19.2)
Hyperbilirubinemia	2 (7.7)	5 (19.2)
Hypokalemia *	2 (7.7)	4 (15.4)
Bone marrow failure	1 (3.8)	4 (15.4)
Activated partial thromboplastin time prolonged	3 (11.3)	4 (15.4)
Staphylococcal infection	0	3 (11.5)
Staphylococcal sepsis	0	3 (11.5)
Hypertriglyceridemia*	3 (11.5)	3 (11.5)

N corresponds to the number of patients in treatment groups
[a] For each Preferred Term patients are included only once even if they experienced multiple events in that preferred term.
[b] Overall study period.
*Pooled Preferred Term as defined in the Statistical Analysis Plan
Source: Table 14.3.1.2.1, Table 14.3.1.28.2; Listing 16.2.7

Serious adverse events and deaths

Death

The most common fatal events were related to disease progression (13.2%) or sepsis/septic shock (5.8%). It should be noted that most studies have followed patients until study completion. In the pivotal Phase II/III study, patients were followed up to 4 months following the last dose of the study drug. This is reflected by the higher incidence of the fatal events in the pooled safety population,

Table 2.7.4- 22 Summary of death regardless of relationship to study drug- Safety population

Preferred Term [a]	Total (N = 189)
At least one adverse events leading to death	62 (32.8)
Disease progression [1]	29 (15.3)
Sepsis *	7 (3.7)
Septic shock	4 (2.1)
Acute respiratory distress syndrome	2 (1.1)
Aspergillus infection *	2 (1.1)
Febrile neutropenia	2 (1.1)
Adenovirus infection	1 (0.5)
Arrhythmia	1 (0.5)
Bacteremia *	1 (0.5)
Bone marrow failure	1 (0.5)
Fungal infection *	1 (0.5)
Interstitial lung disease	1 (0.5)
Intestinal ischemia	1 (0.5)
Meningorrhagia *	1 (0.5)
Metabolic encephalopathy	1 (0.5)
Edema *	1 (0.5)
Pleural effusion	1 (0.5)
Pneumonia	1 (0.5)
Stroke *	1 (0.5)
Subarachnoid hemorrhage	1 (0.5)
Subdural hematoma	1 (0.5)
Tumor lysis syndrome	1 (0.5)

[a] For each preferred term patients are included only once even if they experienced multiple events in that preferred term.
[1] Disease progression is grouping the following PTs: Recurrent leukemia, Multi-organ failure (patient 39-01, GRASPALL SA2 2008 and patient 123-01, GRASPALL 2009-06), Cerebrovascular accident (patient 35-01, GRASPALL GRAALLSA2 2008) and General physical health deterioration (patient 1108-05, GRASPA - AML 2012-01)
*Pooled Preferred Term as defined in the Safety Analysis Plan
Source: Table 2-19 of the Global safety output (Appendix 9).

Study GraspaLL 2009-06

14 fatal events were reported during the study, 2 (7.7%) and 6 (21.4%) with Graspa, L-ASP, and Graspa-s (allergic patient), respectively. Disease progression was the primary cause of death in the 3 arms.

Table 2.7.4- 23 Summary of fatal adverse events regardless of relationship to study drug - Study GRASPALL 2009-06

Preferred Term [a]	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N=26 (%)
At least one SAE leading to death during overall study period	2 (7.7)	6 (21.4)	6 (23.1)
Recurrent leukemia	2 (7.7)	4 (14.3)	1 (3.8)
Septic Shock	0	1 (3.6)	0
Bacteremia	0	0	1 (3.8)
Fusarium infection	0	0	1 (3.8)
Renal failure/acute renal failure	0	1 (3.6)	1 (3.8)
Intestinal ischemia	0	0	1 (3.8)
Cerebral hemorrhage	0	0	1 (3.8)
Acute respiratory distress syndrome	0	0	1 (3.8)
Capillary leak syndrome	0	0	1 (3.8)

N corresponds to the number of patients in treatment groups
[a] For each preferred term patients are included only once even if they experienced multiple events in that preferred term.
Source: Table 9-13 of the CSR

Table 2.7.4- 24 Summary of primary cause of death - Study GRASPALL 2009-06

Primary cause of death	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N=26 (%)
During overall study period			
Disease progression/relapse	2 (7.7)	4 (14.3)	3 (11.5)
Adverse events	0	2 (7.1)	3 (11.5)
Infection	0	2 (7.1)	1 (3.8)
Gastrointestinal toxicity	0	0	1 (3.8)
Post graft toxicity	0	0	1 (3.8)

N corresponds to the number of patients in treatment groups
SAE with fatal outcomes are SAEs recorded until 4 months after the last treatment injection
Source: Table 9-14 of the CSR

Serious Adverse Events

In the safety population, the most common SAEs were consistent with the underlying disease, and included bone aplasia, or disease progression. Serious events of drug hypersensitivity (any drug) occurred in 15 patients (7.9%).

Table 2.7.4- 27 Summary of SAE in ≥ 5% of patients (%) regardless of relationship – ALL studies and Safety population

Preferred Term [a]	Safety population N = 189 (%)	Completed ALL studies N = 102 (%)
At least one serious adverse event	146 (77.2)	91 (89.2)
Sepsis *	33 (17.5)	18 (17.6)
Febrile bone marrow aplasia	32 (16.9)	25 (24.5)
Recurrent leukemia	27 (14.3)	27 (26.5)
Drug hypersensitivity *	15 (7.9)	12 (11.8)
Septic shock	13 (6.9)	12 (11.8)
Febrile neutropenia	12 (6.3)	8 (7.8)
Aspergillus infection *	10 (5.3)	8 (7.8)
Mucosal inflammation	9 (4.8)	9 (8.8)

N corresponds to the number of patients in treatment groups
[a] For each preferred term patients are included only once even if they experienced multiple events in that preferred term.
*Pooled Preferred Term as defined in the Safety Analysis Plan
Note, The seriousness of the following AE is missing: Febrile Aplasia (GRASPALL 2005-01, 020-01)
Source: Table 2.7. and Table 2.8 of the Global safety output (Appendix 9).

Study GraspaLL 2009-06

The most common SAEs were febrile bone marrow aplasia, febrile neutropaenia, and mucosal inflammation, and infection, consistent with the underlying disease.

Table 2.7.4- 28 Summary of SAEs in ≥ 5% of non-allergic patients arranged by MedDRA Preferred Term - Study GRASPALL 2009-06

Preferred Term [a]	GRASPA N = 26 (%)	L-ASP N = 28 (%)
At least one SAE	23 (88.5)	26 (92.9)
Febrile bone marrow aplasia	10 (38.5)	7 (25.0)
Febrile neutropenia	4 (15.4)	5 (17.9)
Mucosal inflammation	3 (11.5)	4 (14.3)
Recurrent leukemia	3 (11.5)	4 (14.3)
Sepsis	3 (11.5)	2 (7.1)
Septic shock	3 (11.5)	1 (3.6)
Staphylococcal sepsis	3 (11.5)	1 (3.6)
Drug hypersensitivity reactions*	3 (11.5)	7 (25.0)
Other allergic reactions*	2 (7.7)	0
Cellulitis	1 (3.8)	2 (7.1)
Pancreatitis*	1 (3.8)	3 (10.7)
Anal abscess	0	2 (7.1)
Lung disorder	0	2 (7.1)

N corresponds to the number of patients in treatment groups
[a] For each Preferred Term patients are included only once even if they experienced multiple events in that preferred term.
*Pooled Preferred Term as defined in the Statistical Analysis Plan
Source: Table 9-11 of the CSR.

Table 2.7.4- 29 Summary of SAEs in ≥ 5% of allergic patients in the GRASPA-s arm arranged by MedDRA Preferred Term - Study GRASPALL 2009-06

Preferred Term [a]	GRASPA-s N=26 (%)
At least one SAE	21 (80.8)
Febrile bone marrow aplasia	5 (19.2)
Febrile neutropenia	3 (11.5)
Mucosal inflammation	5 (19.2)
Drug hypersensitivity *	3 (11.5)
Recurrent leukemia	2 (7.7)
Bone marrow failure	2 (7.7)
Bacteremia	2 (7.7)
Convulsion	2 (7.7)

N corresponds to the number of patients in treatment groups
[a] For each Preferred Term patients are included only once even if they experienced multiple events in that preferred term.
*Pooled Preferred Term as defined in the Statistical Analysis Plan
Source: Table 9- 12 of the CSR

Key Adverse Events of Special Interest

Hypersensitivity reactions

In the safety population a total of 34.9% of the patients presented with hypersensitivity reactions; of these 24.9% were treatment-related (L-asparaginase or non-L-asparaginase). A total of 23 (12.2%) patients had events that were considered related to Graspa. None of these events was fatal; however, these events led to treatment withdrawal in 1 patient. The majority of these events were Grade 1 and Grade 2.

Table 2.7.4- 36 Characteristics of the hypersensitivity reactions - Study GRASPALL 2009-06

Preferred Term*	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N= 26 (%)
N patients with at least any drug or non-drug related AE [a]	7 (26.9)	20 (71.4)	18 (69.2)
N patients with at least any drug-related AE during [a]	2 (7.7)	16 (57.1)	3 (11.5)
Number of events	14	36	33
Number of drug-related# events	2	21	3
Event characteristics, n [1]			
Serious	3 (42.9)	7 (35.0)	3 (16.7)
Drug-related	2 (28.6)	16 (80.0)	3 (16.7)
Leading to withdrawal [b]	0	13 (65.0)	0
Fatal	0	0	0
Drug-related# event characteristics, n [1]			
Serious	2 (100)	6 (37.5)	0
Drug-related	2 (100)	16 (100)	3 (100)
Leading to withdrawal [b]	0	13 (81.3)	0
Fatal	0	0	0
Number of occurrences, n [1]	7	20	18
One	2 (28.6)	11 (55.0)	11 (61.1)
Two or more	5 (71.4)	9 (45.0)	7 (38.9)
Maximum toxicity grade, n [1]			
Grade 1	2 (28.6)	5 (25.0)	6 (33.3)
Grade 2	0	7 (35.0)	7 (38.9)
Grade 3	4 (57.1)	7 (35.0)	4 (22.2)
Grade 4	1 (14.3)	1 (5.0)	1 (5.6)
Action taken, n [2]			
None	2 (14.3)	7 (19.4)	10 (30.3)
Temporarily interrupted	0	1 (2.8)	0
Permanently discontinued	0	13 (36.1)	0
Not applicable**	12 (85.7)	15 (41.7)	23 (69.7)

N corresponds to the number of patients in treatment groups
[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] For each category of events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
Drug-related allergies indicate any allergic event related to L-asparaginase
* All events recorded during the entire study period. Refer to CSR for events during induction only
** Not applicable: when the AE occurred before or after the exposure to study treatment
Source: Table 9-18 of the CSR

Allergic reactions

For completeness, all adverse events considered by the investigators to have allergic nature were grouped under allergic reactions. Those considered drug-induced are grouped under one term – Drug hypersensitivity reactions -, and were the following events: drug hypersensitivity reactions, hypersensitivity, anaphylactic shock, and oedema.

Table 9-16 Summary of number of patients (%) who had at least one reported allergic reaction, regardless of relationship to study drug, by MedDRA Preferred Term - Safety population

Preferred Term [a]	ERY001 N=26 (%)		L-ASP N=28 (%)		ERY001-s N=26 (%)	
	Induction period [b]	Overall Study [c]	Induction period [b]	Overall Study [c]	Induction period [b]	Overall Study [c]
Number of patients with at least one allergic reaction	**3 (11.5)**	**7 (26.9)**	**15 (53.6)**	**20 (71.4)**	**10 (38.5)**	**18 (69.2)**
Drug hypersensitivity	2 (7.7)	4 (15.4)	12 (42.9)	15 (53.6)	8 (30.8)	15 (57.7)
Allergic transfusion reaction	0	2 (7.7)	1 (3.6)	2 (7.1)	0	1 (3.8)
Anaphylactic shock	0	1 (3.8)	1 (3.6)	1 (3.6)	0	2 (7.7)
Transfusion reaction	0	1 (3.8)	0	0	0	1 (3.8)
Angioedema	1 (3.8)	1 (3.8)	0	0	0	0
Rash	0	1 (3.8)	0	0	0	0
Hypersensitivity	0	0	1 (3.6)	3 (10.7)	1 (3.8)	2 (7.7)
Anaphylactic reaction	0	0	1 (3.6)	2 (7.1)	0	1 (3.8)
Erythema multiform	0	0	1 (3.6)	1 (3.6)	0	0
Food allergy	0	0	1 (3.6)	1 (3.6)	0	1 (3.8)
Dyspnea	0	0	0	1 (3.6)	0	0
Allergic dermatitis	0	0	0	0	1 (3.8)	2 (7.7)
Edema	0	0	0	0	1 (3.8)	1 (3.8)
Facial edema	0	0	0	0	1 (3.8)	1 (3.8)

N corresponds to the number of patients in treatment groups
a] For each preferred term patients are included only once even if they experienced multiple events in that preferred term.
[b] AEs during induction period (first 28 days)
[c] AEs during across the overall study period
Source: Table 14.3.1.13.6, Table 14.3.1.13.7, Listing 16.2.7

Allergy to another asparaginase after switch

Allergic reactions following switch to another asparaginase occurred in a total of 9 (16.7%) patients during overall study period, with 4 (15.4%) in ERY001 arm compared to 5 (17.9%) in L-ASP arm. Allergic reactions following switch from ERY001 occurred in 8 (30.8%) patients treated with another asparaginase.

Table 9-19 Number (%) of patients, who had at least one allergic reaction following switch to another asparaginase during overall study period – Safety population

Preferred Term [a]	ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
At least one allergy after switch to another asparaginase	**4 (15.4)**	**5 (17.9)**	**8 (30.8)**
Drug hypersensitivity	3 (11.5)	5 (17.9)	8 (30.8)
Anaphylactic shock	1 (3.8)	0	1 (3.8)

[a] A patient can have one or more preferred term (PT) reported under a given system organ class (SOC).
Source Table 14.3.1.23, Listing 16.2.7.

Pancreatic events

22.2% of the patients had pancreatic AEs in the safety population. The majority of these events were drug-related (34 (18%) patients), and comprised elevated amylase and/or lipase, without manifestations of clinical pancreatitis. Four patients (2.1%) had SAEs, but none of these events was fatal. Grade 1 and 2 rates were generally similar to Grade 3 and 4.

Table 2.7.4- 37 Characteristics of the pancreatic events – Safety population

Preferred Term * [a]	Safety population N= 189 (%)
Number of patients with pancreatic events [a]	**42 (22.2)**
Biochemical pancreatitis	39 (20.6)
Pancreatitis	6 (3.2)
Event characteristics, n [1]	
At least one serious event	4 (9.5)
At least one drug-related event	34 (80.9)
At least one AE leading to withdrawal [b]	1 (2.4)
Fatal event	0
Maximum toxicity grade, n [1]	**42**
Grade 1	17 (40.5)
Grade 2	7 (16.7)
Grade 3	15 (35.7)
Grade 4	3 (7.1)
Outcome, n [2]	**42**
Resolved	42 (100.0)
Action taken, n [2]	**42**
None	33 (78.6)
Temporarily interrupted	1 (2.4)
Permanently discontinued	1 (2.4)
Not applicable**	7 (16.7)

[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] For each category of events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
*All events recorded during the entire study period.
** Action taken is 'not applicable' when the AE occurred before or after the exposure to study treatment
Source: Table 2-35 of Global safety output (Appendix 9).

Table 2.7.4- 38 Characteristics of the pancreatic events - Study GRASPALL 2009-06

Preferred Term*	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N=26 (%)
Number of patients with pancreatic events [a]	**9 (34.6)**	**16 (57.1)**	**10 (38.5)**
Biochemical pancreatitis	9 (34.6)	16 (57.1)	10 (38.5)
Amylase increased	8 (30.8)	8 (28.6)	6 (23.1)
Lipase increased	7 (26.9)	10 (35.7)	7 (26.9)
Amylase and lipase elevation	6 (23.1)	6 (32.1)	5 (23.1)
Clinical pancreatitis	2 (7.7)	4 (14.3)	1 (3.8)
Number of events	**30**	**37**	**22**
Event characteristics, n [1]			
Serious	1 (11.1)	4 (25.0)	1 (10.0)
Drug-related	7 (77.8)	15 (93.8)	7 (70.0)
Leading to withdrawal [b]	0	1 (6.3)	0
Fatal	0	0	0
Maximum toxicity grade, n [1]			
Grade 1	1 (11.1)	3 (18.8)	2 (20.0)
Grade 2	2 (22.2)	1 (6.3)	1 (10.0)
Grade 3	3 (33.3)	7 (43.8)	7 (70.0)
Grade 4	3 (33.3)	5 (31.3)	0
Outcome, n [2]			
Resolved	24 (100)	32 (97.0)	18 (100)
Not resolved	0	1 (3.0)	0
Action taken, n [2]			
None	21 (70.)	22 (59.5)	14 (63.6)
Temporarily interrupted	0	2 (5.4)	0
Permanently discontinued	0	1 (2.7)	0
Not applicable**	9 (30.0)	12 (32.4)	8 (38.4)

N corresponds to the number of patients in treatment groups
[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] For each category of coagulation disorders events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
* All events recorded during the entire study period. Refer to CSR for events during induction only
** Action taken is 'not applicable' when the AE occurred before or after the exposure to study treatment
Source: Table 9-21 of the CSR

Coagulation disorders

A total of 39.2% of the patients presented with impaired coagulation parameters. In 19 (10.1%) patients, these events were serious, and of those, 4 patients had a fatal outcome. Study drug-related AEs accounted for 6.3% of the patients, which is considered lower than the reported events with other L-asparaginase formulations. None of the events was fatal, but in one patient in the Graspa arm, the event led to treatment withdrawal.

Table 2.7.4- 40 Characteristics of the coagulation disorders - Study GRASPALL 2009-06

Preferred Term*	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N=26 (%)
Number of patients with at least AE [a]	**12 (46.2)**	**25 (89.3)**	**18 (69.2)**
Impaired coagulation parameters	11 (42.3)	24 (85.7)	18 (69.2)
Hypofibrinogenemia	8 (30.8)	19 (67.9)	10 (38.5)
Anti-thrombin III decreased	4 (15.4)	20 (71.4)	9 (34.6)
Activated PTT prolonged	1 (3.8)	4 (14.3)	4 (15.4)
Prothrombin level decreased	0	3 (10.7)	0
Thrombotic/embolic/hemorrhagic events	3 (11.5)	4 (14.3)	2 (7.7)
Event characteristics, n [1]			
Serious	1 (5.6)	2 (8.0)	2 (16.7)
Drug-related	9 (50.0)	23 (92.0)	10 (83.3)
Leading to withdrawal [b]	1 (5.6)	0	0
Fatal	0	0	1 (6.7)
Maximum toxicity grade, n [1]			
Grade 1	2 (16.7)	2 (8.0)	2 (11.1)
Grade 2	2 (16.7)	2 (8.0)	3 (16.7)
Grade 3	7 (58.3)	14 (56.0)	12 (66.7)
Grade 4	1 (8.3)	7 (28.0)	0
Grade 5	0	0	1 (5.6)
Action taken, n [2]			
None	11 (55.0)	70 (81.4)	15 (51.7)
Temporarily interrupted	0	3 (3.5)	0
Permanently discontinued	1 (5.0)	0	0
Not applicable**	8 (40.0)	13 (15.1)	14 (48.3)

N corresponds to the number of patients in treatment groups
[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] PT: Preferred Term For each category of coagulation disorders events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
** All events recorded during the entire study period. Refer to CSR for events during induction only
* Action taken is 'not applicable' when the AE occurred before or after the exposure to study treatment
Source: Table 9-20 of the CSR

Hepatic events

Hepatic toxicity was not generally well defined in all studies, and generally relied on the investigator's assessment. Hepatic toxicities comprised events such as high concomitant elevations of AST, ALT and other hepatic function test, Grade 3 or 4 hyperbilirubinaemia, and/or clinical evidence of hepatic failure. These events collectively occurred in 25.4% of the patients. In 4 (5.2%) patients, these events were serious, leading to treatment withdrawal in 2 patients. Concomitant elevation of AST and/or ALT with hyperbilirubinaemia occurred in 3.8%, 14.3%, and 15.4% of the patients in the Graspa arm, L-ASP arm, and Graspa-s arm, respectively.

Table 2.7.4- 43 Characteristics of hepatic events – Safety population

Preferred Term* [a]	Safety population N= 189 (%)
Number of patients with hepatic events [a]	**77 (40.7)**
Transaminases increased	49 (25.9)
Hepatotoxicity	48 (25.4)
Transaminases and bilirubin increased	11 (5.8)
Event characteristics, n [1]	**77**
At least one serious event	4 (5.2)
At least one drug-related event	44 (57.1)
At least one AE leading to withdrawal [b]	2 (2.6)
Fatal event	0
Maximum toxicity grade, n [1]	**77**
Grade 1	11 (14.3)
Grade 2	11 (14.3)
Grade 3	44 (57.1)
Grade 4	11 (14.3)
Action taken, n [2]	**77**
None	55 (71.4)
Permanently discontinued	1 (1.3)
Not applicable**	21 (27.3)

[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] For each category of events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
*All events recorded during the entire study period.
** Action taken is 'not applicable' when the AE occurred before or after the exposure to study treatment
Source: Table 2-38 of the Global safety output (Appendix 9)

Table 2.7.4- 44 Characteristics of hepatic events - Study GRASPALL 2009-06

Preferred Term*	GRASPA N=26 (%)	L-ASP N=28 (%)	GRASPA-s N=26 (%)
Number of patients with events [a]	19 (73.1)	18 (64.3)	17 (65.4)
Transaminase increased	15 (57.7)	12 (42.9)	12 (46.2)
Transaminase and bilirubin increased	1 (3.8)	4 (14.3)	4 (15.4)
GGT increased	6 (23.1)	10 (35.7)	8 (30.8)
Hepatotoxicity	3 (11.5)	6 (21.4)	8 (30.8)
Event characteristics, n [1]			
Serious	2 (10.5)	1 (5.6)	1 (5.9)
Drug-related	6 (31.6)	13 (72.2)	7 (41.2)
Leading to withdrawal [b]	0	1 (5.6)	0
Fatal	0	0	0
Maximum toxicity grade, n [1]			
Grade 1	1 (5.3)	0	0
Grade 2	0	3 (16.7)	2 (11.8)
Grade 3	15 (78.9)	12 (66.7)	5 (29.4)
Grade 4	3 (15.8)	3 (16.7)	10 (58.8)
Action taken, n [2]			
None	27 (51.9)	31 (63.3)	25 (49.0)
Permanently discontinued	0	1 (2.0)	0
Not applicable**	25 (48.1)	17 (34.7)	26 (51.0)

N corresponds to the number of patients in treatment groups
[1] Analysis is performed on the number of patients with at least one event
[2] Analysis is performed on the total number of events
[a] For each category of coagulation disorders events patients are included only once even if they experienced multiple events in that category
[b] Leading to withdrawal: Adverse event with the action on the study treatment 'Permanently discontinued'
*All events recorded during the entire study period. Refer to CSR for events during induction only
** Action taken is 'not applicable' when the AE occurred before or after the exposure to study treatment
Source: Table 9-23 of the CSR.

Transfusion reactions

In the entire safety population, there was a total of 11 (5.8%) patients who had evidence of transfusion reactions; of these 5 (4.9%) were encountered in patients with ALL. Of the 11 patients, 4 (7.4%) were children and 7 (5.2%) were adults. Of these events, 3 (1.6%) were Grade 3 and 4. Two (1.1%) of the 11 patients had events there were considered serious. None of the events was fatal. None of the events was considered related to Graspa. The proposed label provides a precaution regarding patients receiving blood transfusion between Graspa subscriptions and its administrations, in which new compatibility tests should be performed.

Laboratory findings

Study GraspaLL 2009-06

Haematology

Most haematologic abnormalities had a similar incidence between the two treatment groups, and were generally reflective of this patient population undergoing intense therapy for ALL.

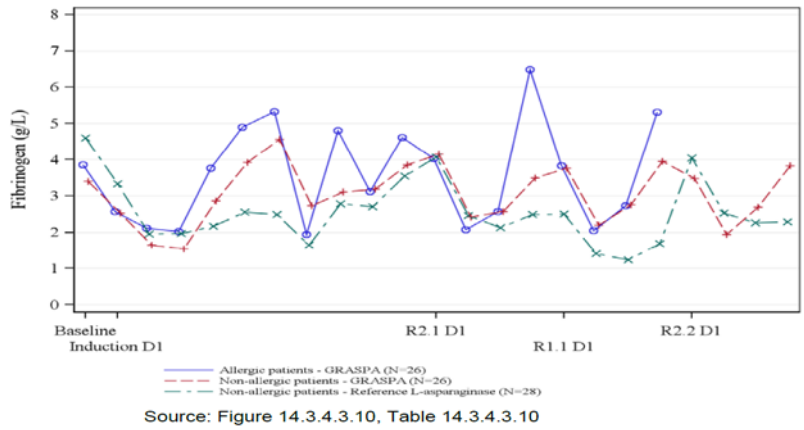
Coagulation

PTT prolongation was less marked with ERY001 compared to L-ASP treatment group. For example, the mean (SD) PTT value at F2/Day 27 was 38.1 (SD: 7.8) seconds with ERY001 compared to 42.7 (SD: 11.7) seconds with L-ASP. Similarly, the mean (SD) PTT value at VANDA/DAY 27 was 45.2 (SD: 17.9) seconds, compared to 86.6 (SD: 91.7) seconds in the L-ASP arm, Figure 9-7.

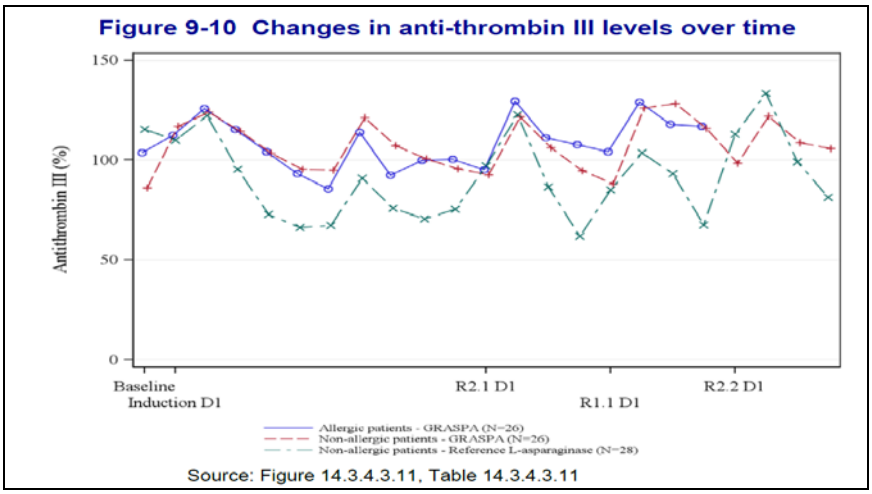
There were no clinically significant differences in the % changes of PT between the ERY001 and L-ASP arms.

Plasma fibrinogen values and anti-thrombin III activity varied over time during study treatment period in all treatment groups, with considerable decrease of these parameters in L-ASP arm, particularly during induction phase.

Figure 9-9 Changes in fibrinogen levels over time



Source: Figure 14.3.4.3.10, Table 14.3.4.3.10



Serum biochemistry

Baseline values of AST were within the normal ranges in 68.4% and 64.7% in ERY001 and L-ASP, respectively. The maximum mean values in ERY001 arm were 161.2 (223.8) IU/L observed at F1-D10, compared to 539.2 (SD: 1553.9) IU/L in L-ASP arm observed at F2-D24. Similarly, AST levels were within normal range in 56.3% in ERY001-s arm. The maximum mean (SD) values were 279.2 (SD: 436.6) IU/L, observed at F1-D4.

Similar pattern of elevated ALT was also observed, with the highest mean) value of 388.2 (SD: 594.8) IU/L and 147.7 (SD: 237.0) IU/L at F1/day10 in ERY001 and L-ASP arms, respectively. In the ERY001-s arm, 43.8% of patients had abnormal values at baseline, with mean ALT level of 64.6 (SD: 65.0) IU/L. An increase in ALT values was observed, with the highest mean ALT value of 670.3 (SD: 531.5) IU/L observed at F1-D10.

The mean serum bilirubin at baseline was within the normal ranges in all treatment groups. However, 17.6% and 11.1% of patients has abnormal values in ERY001 and L-ASP arms, respectively. At F2-D24, peak mean levels were 8.4 (SD: 5.9) µmol/L and 42.8 (SD: 106.1) µmol/L, in ERY001 and L-ASP arms, respectively. In the ERY001-s arm, the peak mean bilirubin level was 15.3 (SD: 10) µmol/L,

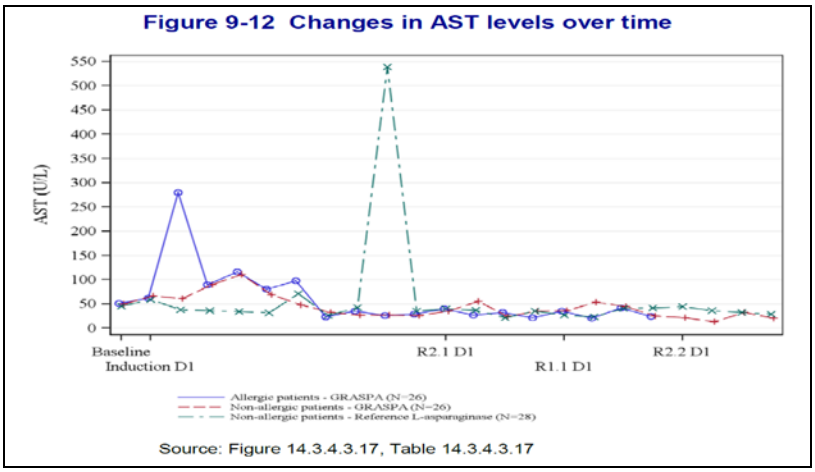
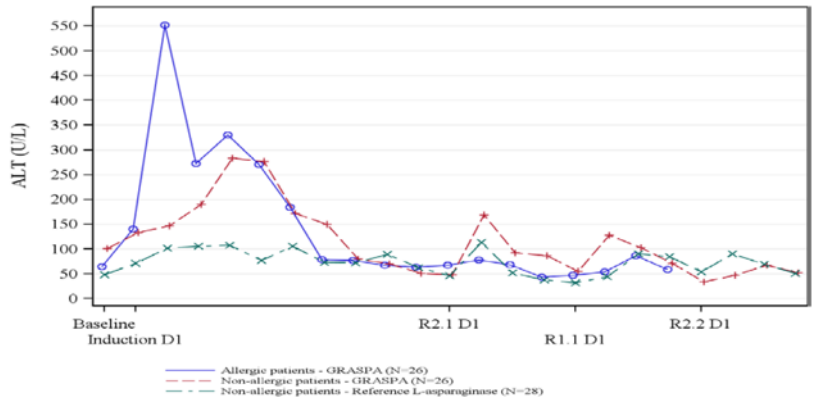
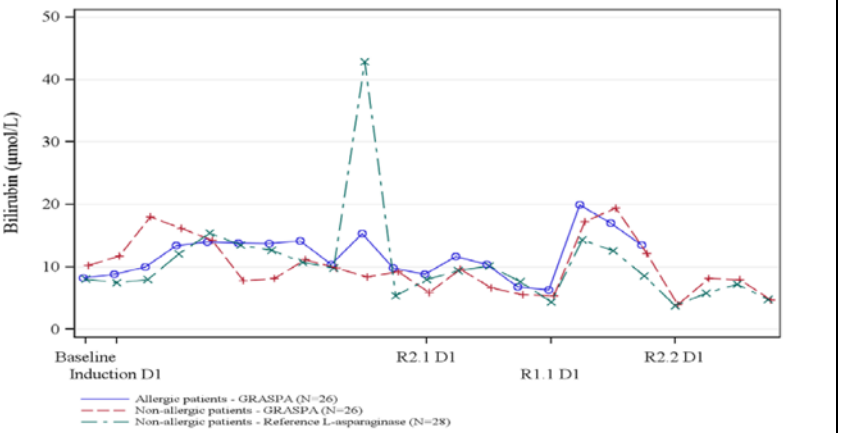


Figure 9-13 Changes in ALT levels over time



Source: Figure 14.3.4.3.18, Table 14.3.4.3.18

Figure 9-14 Changes in bilirubin levels over time



Source: Figure 14.3.4.3.22, Table 14.3.4.3.22

Pancreatic Enzymes (Amylase and Lipase)

The number of patients assessed for amylase and lipase was highly variable within each treatment group. Thus in ERY001 arm, only 4 patients had baseline amylase values and up to 13 patients were assessed during the study. Only 3 patients in L-ASP arm had baseline amylase assessment, and up to 13 patients were assessed during the study. Values of amylase activity varied considerably between approximately 40 and 80 IU/L over time in all treatment groups, with a trend towards higher mean values in the L-ASP arm.

Similar pattern was also observed with serum lipase.

Safety in special populations

Age

In the age groups [55-65[, and ≥ 65 years, toxicities known to be associated with L-asparaginase occurred at greater proportions than their younger counterparts, and included impaired coagulation parameters (74.2%), hyperbilirubinaemia (35.5%), elevated transaminases (38.7%), and pancreatitis (38.7%). In contrast, there was relatively lower incidence of myelosuppression, which reflects the limited used of multi-agent intensive chemotherapy, more aggressive disease, poor prognosis, concomitant comorbidities and poor body reserves. The most common SAEs were myelosuppression in children, which reflects the use of intensive chemotherapy for induction remission, which is used universally in the paediatric population. On the other hand, SAEs due to infection, and disease

progression were more common in adult population, which reflects poorer prognosis, presence of comorbidities and overall disease burden.

MedDRA Terms	Age <65* number (percentage)	Age 65-74** number (percentage)	Age 75-84** number (percentage)	Age 85+** number (percentage)
Total AEs***	1056	319		-
Serious AEs – Total***	135	49	8	-
- Fatal	30	13	3	-
- Hospitalization/prolong existing hospitalization	127	23	3	-
- Life-threatening	9	10	1	-
- Disability/incapacity	1	0	0	-
- Other (medically significant)	39	3	1	-
AE leading to drop-out [a]	2	3	1	-
Psychiatric disorders [b]	1	0	0	-
Nervous system disorders[b]	9	0	0	-
Accidents and injuries [b]	6	0	0	-
Cardiac disorders[b]	2	1	0	-
Vascular disorders[b]	9	1	2	-
Cerebrovascular disorders[b]	1	1	0	-
Infections and infestations[b]	91	15	1	-
Anticholinergic syndrome[b]	8	1	0	-
Quality of life decreased	Not available	Not available	Not available	-
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures[b]	5	0	0	-
<other AE appearing more frequently in older patients>[b]	None	None	None	-

Gender, Race, and Weight

The effect of gender, race or weight on Graspa pharmacokinetics has not been directly examined. However, there was no evidence to suggest a different safety profile in patients of different gender, race or weight, based on other approved formulations.

Hepatic insufficiency

No specific studies have been conducted to examine AEs in patients with hepatic insufficiency. The no need for dose adjustments in the case of hepatic impairment is considered adequately justified. In the current context of common events of hepatotoxicity and liver enzymes elevations, which may be severe, the need for a clear warning is agreed upon. In addition, a cautionary statement has been included in Section 4.2 of the SmPC, which requires some rewording to properly advice clinicians

However, the current warning on hepatic toxicity is considered insufficient to properly advice clinicians. Available data should be included. In addition, the current recommendation for treatment discontinuation in case of BR levels >3 or transaminase levels >10 times ULN requires further justification. The Applicant agrees to include appropriate information in the SmPC concerning the risk for hepatotoxicity, which in principle is in line with that of available L-asparaginase formulations. Section 4.3 of the SmPC has been updated with a contraindication for patients with severe hepatic impairment (bilirubin > 3 times upper limit of normal [ULN]; transaminases > 10 times ULN). In addition the section 4.4 (Special warnings and precautions of use) of the SmPC already describes precaution for hepatotoxicity: Hepatic enzymes should be monitored before and regularly during treatment with Graspa. Treatment with eryaspase should be discontinued in the event of bilirubin levels >3 times upper limit of normal [ULN] or transaminase levels >10 times ULN.This is acceptable.

Renal impairment

No specific studies have been conducted to examine AEs in this population. Eryaspase is not excreted in urine, so that renal impairment is not expected to affect clearance and thus, no particular treatment recommendations are needed in these patients. Appropriate information is included in the SmPc

Immunological events

Immunogenicity

Pivotal Study GraspaLL 2009-06

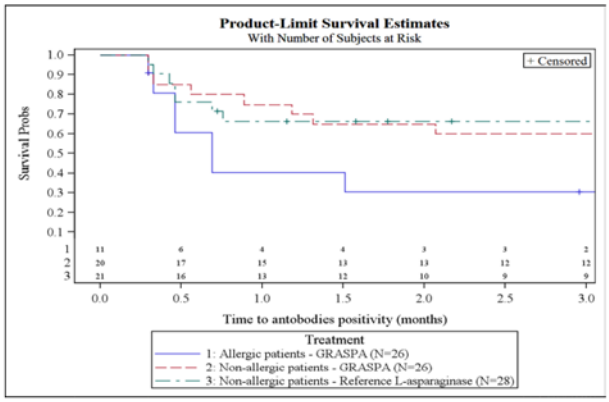
At study entry, a total of 6 (23.1%), 7 (25.0%), and 15 (57.7%) patients had positive L-ASP antibodies in the ERY001, L-ASP, and ERY001-s arms. At the end of treatment exposure, conversion to positive antibody status occurred in 8 (30.8%) patients treated with ERY001, 7 (25.0%) patients treated with L-ASP, and 7 (26.9%) patients in the ERY001-s arm.

Table 8-35 Summary of anti-L-asparaginase antibodies over time – Treated analysis set

		ERY001 N=26 (%)	L-ASP N=28 (%)	ERY001-s N=26 (%)
Baseline antibody status	Negative	20 (76.9)	21 (75.0)	11 (42.3)
	Positive	6 (23.1)	7 (25.0)	15 (57.7)
Antibody status during treatment exposure	Remain negative	12 (46.2)	14 (50.0)	4 (15.4)
	Remain positive	6 (23.1)	6 (21.4)	13 (50.0)
	Positive conversion	8 (30.8)	7 (25.0)	7 (26.9)
	Median time (mo)	.	.	0.6
	Negative conversion	0	1 (3.6)	1 (3.8)

N corresponds to the number of patients in treatment groups

Figure 8-10 Kaplan-Meier plot of time to positive antibody conversion – Treated analysis set



Source: Figure 14.2.2.8.9

GraspaLL/GRAALL SA2-2008 Study

All patients were negative for anti-L-asparaginase antibodies at inclusion, and on the day following the first Graspa administration. At the end of induction 1, two patients (7.4%) presented positive antibodies, one in the 100 IU/kg and one in the 150 IU/kg group. One month later, at the end of induction 2, ten patients (45.5%) had positive antibodies: 5 patients (55.6%) treated at 150 IU/kg, 4 patients (36.4%) at 100 IU/kg and 1 patient (50%) at the lowest dose. Some of these positive results were previously considered as doubtful (neither negative nor positive), and became positive at the end of induction 2.

Table 26. Summary of anti-L-asparaginase antibodies over time (Analysis Set)

		Treatment dose Number (%) of patients			
		50 IU/Kg	100 IU/Kg	150 IU/Kg	Total
Anti L-Asp antibodies					
Induction 1					
D3	N	3	13	14	30
	Negative	3 (100.0%)	13 (100.0%)	14 (100.0%)	30 (100.0%)
D4	N	3	13	14	30
	Negative	3 (100.0%)	13 (100.0%)	14 (100.0%)	30 (100.0%)
D22 to D28	N	2	13	12	27
	Doubtful	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (3.7%)
	Negative	2 (100.0%)	11 (84.6%)	11 (91.7%)	24 (88.9%)
	Positive	0 (0.0%)	1 (7.7%)	1 (8.3%)	2 (7.4%)
Induction 2					
D6	N		1	6	7
	Doubtful		0 (0.0%)	1 (16.7%)	1 (14.3%)
	Negative		1 (100.0%)	4 (66.7%)	5 (71.4%)
	Positive		0 (0.0%)	1 (16.7%)	1 (14.3%)
D22 to D28	N	2	11	9	22
	Doubtful	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (4.5%)
	Negative	1 (50.0%)	6 (54.5%)	4 (44.4%)	11 (50.0%)
	Positive	1 (50.0%)	4 (36.4%)	5 (55.6%)	10 (45.5%)

Discontinuation due to AEs

Pivotal Study GraspaLL 2009-06

In this study, the proportion of patients who interrupted or permanently discontinued treatment was lower in the Graspa arm (3.8% each) than in the L-ASP arm (28.6% and 53.6%, respectively).

Drug hypersensitivity reactions were the main reason for discontinuation in the L-ASP arm (35.7%).

In the Graspa-s arm (allergic patients), 1 (3.8%) patient each interrupted or permanently discontinued treatment with Graspa, which was attributed to mucosal inflammation and infection.

Table 2.7.4- 32 Number (%) of patients with at least one AE leading to temporary or permanent discontinuation of study medication during overall study period - Study GRASPALL 2009-06

	GRASPA N=26 (%)		L-ASP N=28 (%)		GRASPA-s N=26 (%)	
	Interr[b]	stop	Interr [b]	stop	Interr [b]	stop
At least one AE	1 (3.8)	1 (3.8)	8 (28.6)	15 (53.6)	1 (3.8)	1 (3.8)
Drug hypersensitivity	0	0	0	10 (35.7)	0	0
Hypersensitivity	0	0	0	2 (7.1)	0	0
Anti-thrombin III decreased	0	0	2 (7.1)	0	0	0
Pancreatic enzymes increased	0	0	2 (7.1)	0	0	0
Partial seizures	1 (3.8)	0	0	0	0	0
Pulmonary embolism	0	1 (3.8)	0	0	0	0
Febrile bone marrow aplasia	0	0	1 (3.6)	0	0	0
Hypofibrinogenemia	0	0	1 (3.6)	0	0	0
Anaphylactic shock	0	0	0	1 (3.6)	0	0
Anaphylactic reaction	0	0	1 (3.6)	0	0	0
Cellulitis	0	0	1 (3.6)	0	0	0
Hepatotoxicity	0	0	0	1 (3.6)	0	0
Transaminase increased	0	0	0	1 (3.6)	0	0
Subcutaneous abscess	0	0	1 (3.6)	0	0	0
Mucosal inflammation	0	0	1 (3.6)	0	1 (3.8)	1 (3.8)
Infusion site thrombosis	0	0	1 (3.6)	0	0	0
Septic arthritis	0	0	0	0	0	1 (3.8)
Depression	0	0	0	0	0	1 (3.8)

N corresponds to the number of patients in treatment groups
[a] A patient can have one or more preferred term (PT) reported under a given Preferred Term
[b] interr: interrupted
Source: Table 9-15 of the CSR

3.3.6 Discussion and conclusion on clinical safety

The safety profile of Graspa as combination therapy with other treatments has been evaluated in a total of 189 patients with ALL, AML or solid tumours in 8 completed or ongoing ERYTECH sponsored clinical studies in Europe and US, as of the clinical cut-off date of 2nd March 2015. Of them, 114 patients received Graspa in completed clinical studies, with 77 receiving the licensed dose and 54 with the opportunity to receive more than one dose at the proposed licensed dose. Of them, a total of 102 patients constitute the ALL population from completed studies, which comprises Study GraspaLL 2009-06, GraspaLL 2005-01, and Study GraspaLL GRAALL SA2 2008; the narratives from 10 patients included in the EAP (Study GraspaLL 2012-10 EAP), which started in 2014 as a multicentre open-label study, are also reported. Total patient numbers in the Graspa ALL programme are small even with the orphan status of the disease taken into consideration.

Treatment compliance and total adherence to treatment was high in the main ALL studies, particularly in the Graspa treated arm. This is not unexpected, given the more convenient regimen as compared to L-asp. By contrary, there was a high rate of study treatment discontinuations across the studies conducted, with lack of efficacy being the main reason within the ALL studies. In the pivotal study, the main reasons for treatment discontinuation were: target levels of asparagine depletion not reached in 16 (61.5%) patients in the Graspa arm, and adverse events reported in 15 (53.6%) patients in the L-ASP arm. In the Graspa-s arm, of the 26 patients enrolled, 10 (38.5%) patients completed induction treatment. The main reason for discontinuing treatment was target level of asparagine depletion not reached during induction and post-induction phase (n=15 [57.7%] patients), consistent with results in the controlled arm. Discontinuations due to adverse events are relatively low during treatment with Graspa. However, given that Graspa is not administered on a daily basis but every 2-3 weeks, discontinuation is not always a feasible/appropriate measure to e.g. revert a potential AE and this may explain to an extent the lower rate of discontinuations due to AEs as compared to L-asparaginase treated arm.

According to the Applicant, the patient population enrolled in the key pivotal and supportive studies is representative of patients with first-line, refractory or relapsed ALL. However, main evidence provided

belong to a relapsed setting and an overall fit children-adult population, while data in first line is limited to elderly patients, with no comparison over available treatment options. The claimed indication is for relapsed patients, therefore, this is not considered a major issue as long as this is clearly stated in the wording of the indication. Furthermore, only patients after a first relapse have been studied, whilst patients with 2nd, 3rd relapse were excluded from participation as were patients with Chromosome Ph+ patients. These limitations are properly reflected in the SmPC.

Baseline demographic characteristics from pivotal and main supportive studies in ALL show that 60% of patients included were male, median age 20.7 yrs (2; 78), with the following age distribution: 1-11 yrs: 37 patients (36%), 11-18: 10 patients (10%), 18-55: 24 (23.5%), 55-65: 12 (11.8%) and ≥65 yrs 19 (18.6%). In total 46% were paediatric patients vs 53.9% adults. These are mainly driven by two distinct subset of patients: those from the pivotal study, mostly paediatric patients with a prior relapse, for which the demographic characteristics were similar between the treatment groups and were generally consistent with the characteristics expected of this patient population and those from the supportive study 2005-08 conducted in naïve elderly patients, with only 3 patients over 75 years. The limited information in patients over 65 yrs is reflected in the SmPC.

In the 3 key studies (pivotal Phase II/III and supportive studies), all patients experienced at least one AE; of these 80.4% had related AE. The majority of the events resolved, with 15.7% of the patients reported AEs leading to either temporary or permanent discontinuation of the study drug. The majority of the patients (89.2%) experienced at least one SAE, with 27.5% reporting SAEs that were considered study-drug related. The related SAEs with a fatal outcome accounted for 3.9%. In safety population of completed ALL studies, AE rate was similar in children and adults. However, adult population (N= 55) had a higher rate of events leading to study drug discontinuation (23.6% vs. 6.4%), related SAEs (41.8% vs. 10.6%), SAEs with fatal outcome (65.5% vs. 17.0%), and related SAEs with fatal outcome (7.3% vs. 0%). This pattern of higher rates in adults, especially older adults compared to children, reflects the presence of comorbidities in this population, and is not unexpected. The SmPC includes a recommendation to pay particular attention to the existing co-morbidities before starting treatment in patients over 55 years old (Section 4.2 SmPC).

The most commonly reported AEs were myelosuppression, impaired coagulation, hepatotoxicity, pancreatic toxicity, hyperglycaemia, and drug hypersensitivity, infections/sepsis, which are common known AEs associated to L-asparaginase treatment in the ALL population. Higher AE rates in patients administered the 150 IU/kg than those administered the lower doses were also seen. Information in the SmPC has been updated to properly reflect these risks. Pancreatitis and history of pancreatitis due to L-asparaginase has been included as contraindications for the use of Graspa, which is in line with SmPC of recently authorised asparaginase formulations.

Although the number of patients in the development programme was small, as were the numerical differences in AE rates between study cohorts, for almost all asparaginase AEs of interest incidence was lower in the non-asparaginase allergic Graspa arm than in the L-asparaginase arm. These events included reduced antithrombin III, hypofibrinogenaemia, hyperglycaemia, clinical and biochemical pancreatitis, hypoalbuminaemia, elevated GGT and prolonged APTT. No differences in PT or TT AEs were stated. Interestingly, in the Graspa arm AST and ALT, as adverse events occurred more frequently than in the L-asparaginase arm. However, hepatotoxicity events were not increased. Further, it is reassuring that elevated GGT and hyperbilirubinaemia AEs occurred less frequently in the Graspa arm. The higher frequency of elevated AST/ALT reports may be due to concomitantly administered cytotoxic agents or could be a chance finding. The asparaginase related AEs of interest in the asparaginase allergic Graspa study arm occurred with similar frequency to those observed in the non-allergic arm. However, slightly more hepatotoxicity and reduced anti-thrombin III events occurred.

There were no differences in death rates in any of the studies between Graspa and L-asparaginase study arms. The majority of deaths were related to disease progression. The AEs leading to death (cerebrovascular accident and metabolic encephalopathy) occurred in the highest dose group, 150 IU/kg. Follow-up was for 4 months after the last treatment injection, which is satisfactory as the majority of erythrocytes persist for 120 days in the circulation. Follow-up was therefore sufficiently long to capture all deaths and adverse events which were at least possibly related to study drug.

The majority of SAEs were due to the effects of concomitantly administered cytotoxic therapy and to disease progression. SAEs at least possibly attributable to asparaginase therapy were seen in smaller numbers. However apart from serious hypersensitivity reactions, which occurred with slightly higher incidence in the L-asparaginase arm, no differences could be seen between Graspa and L-asparaginase administered patients or between patients administered different doses of Graspa.

As discussed for AEs in general in the report, the majority of asparaginase related adverse events of interest occurred with greater frequency in the L-asparaginase arm than in the Graspa study arms. This is also true of the laboratory data, which showed this trend, even when broken down to severity category. As with AEs, incidence of elevated AST and ALT values was the exception to this, where rates in those administered Graspa were higher than in the control group. However, hepatic events, defined as incidence of simultaneously elevated AST/ALT, bilirubin and/or evidence of hepatic failure, did not follow this trend. Here too rates were higher in the L-asparaginase arm than in the non-allergic Graspa arm. Currently the changes in AST/ALT are not thought to constitute evidence of a specific effect of Graspa on the liver, which is theoretically possible as erythrocytes are removed from the circulation, mainly through the reticuloendothelial system, much of which is housed in the liver. However, a direct hepatotoxic effect resulting from elimination of Graspa loaded erythrocytes cannot be excluded.

Results were more variable in the study arm into which were recruited Graspa subjects with known hypersensitivity to asparaginase. Occasionally, results suggested similar toxicity to Graspa e.g. with regard to pancreatic event results. However for the most part, adverse event rates approached those of the L-asparaginase arm (hepatotoxicity, hypersensitivity, allergy, coagulation), suggesting that even the non-allergy toxicity profile of Graspa in patients with known allergy to asparaginase is noticeably worse than in patients without previous allergy.

The main changes of interest in laboratory values were the prolonged PT and APTT values, higher rates of hyperglycaemia and lower plasma concentrations of fibrinogen and antithrombin III in patients administered L-asparaginase compared to Graspa administered patients. These results are in line with the reported AE rates of hyperglycaemia and abnormal coagulation.

As expected, due to the correlation between asparaginase allergy and anti-drug antibody (ADA) positivity, at baseline, approximately half of patients in the asparaginase allergic arm were ADA positive. The baseline ADA rates in the non-allergic study arms were half that. Interestingly, immunogenicity data in study GraspaLL 2009-06 did not suggest any differences between Graspa and L-asparaginase in the three study arms, in that rates of positive conversion were similar. Given that the Applicant has admitted that the ADA assay has not been adequately validated, these data are not considered reliable.

Reliable data about immunogenicity should be provided. New analyses of the samples from the pivotal trial will be provided and timelines should be specified.

Overall, the toxicity profile of Graspa appears to be improved compared to that of L-asparaginase, both in terms of the rate and severity of asparaginase associated AEs. For the AE of principal concern - hypersensitivity reactions - this may well be attributed to the sequestering of native asparaginase within erythrocytes, limiting direct exposure to the immune system components implicated in these

reactions. It is odd therefore that the immunogenicity data should be similar for both Graspa and L-asparaginase study arms. Firm conclusions cannot currently be drawn on these data as the immunogenicity data are not currently considered robust. Whilst it is of great benefit to have an asparaginase with a significantly improved safety profile, the main concern is that this is at the expense of efficacy, as most of asparaginase–related AEs result from lengthy periods of asparagine depletion, the same mechanism through which asparaginases exert their anti-leukaemic effect. The inferior effect of Graspa on asparaginase depletion (see Efficacy discussion) is further evidence of this hypothesis.

The lack of dedicated DDI studies is considered acceptable. However, the decrease in serum proteins caused by L-asparaginase formulations, including Graspa, can increase the toxicity of other medicinal products that are protein bound. This has been reflected in the SmPC. In addition, the Applicant has updated information in the SmPc concerning the risk to disturb the MoA of other substances which require cell division for their effect, e.g. methotrexate, cytarabine, etc.

Information on the risk for DDI with other drugs which may alter coagulation is not considered appropriate (SmPC 4.5). A new wording in line with the following should be included: “The use of Graspa can lead to fluctuating coagulation factors. This can promote the tendency to bleeding and/or thrombosis. Caution is therefore needed when anticoagulants such as coumarin, heparin, dipyridamole, acetylsalicylic acid or nonsteroidal anti-inflammatory drugs are given concomitantly.” In addition, the Applicant should discuss to what extent alterations in coagulation parameters can be pronounced when prednisone and Graspa are given at the same time, (e.g. fall in fibrinogen and Antithrombin III deficiency, ATIII). The need to reflect this in the SmPC should be discussed. ERYTECH do agree to add the following contraindications in the section 4.3 in the SmPC: *Pre-existing known coagulopathy (e.g. haemophilia) and a history of, serious haemorrhage or serious thrombosis with prior asparaginase therapy. M*oreover, it is specified in section 4.4 (Special warnings and precautions for use) of the SmPC, that Blood count and clotting profile should be monitored before and during treatment with Graspa. Information is Section 4.5 has been updated to be aligned with other L-asp formulations. This is acceptable.

The applicant has agreed to to inform on the risk of concomitant treatments with medicinal products associated to HS reactions, i.e. vincristine. The following has been added in the section 4.5 of the SmPC:

Vincristine

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.

Due to known risks for hepatotoxicity associated to Graspa, there is a potential risk that this may impair the hepatic clearance of oral contraceptives. A proposal to reflect this in the SmPC (Section 4.4, 4.5 and 4.6) has been made and is considered acceptable.

3.3.7 Conclusions on clinical safety

From a safety point of view there are no major concerns. Graspa appears to provide an alternative to current asparaginase products with a better safety profile compared to authorised products. Indeed, in the pivotal trial, most well-known toxicities of asparaginase were less frequent with Graspa than with L-ASP: not only hypersensitivity reactions (as expected since asparaginase is mostly encapsulated) but also abnormal coagulation factors and parameters, abnormal pancreatic and liver enzymes (except for transaminases), hypoalbuminaemia, hyperglycaemia, as well as hepatotoxicty, pancreatitis, and thrombotic/haemorrhagic events. Although the precise pathogenesis of these latter toxicities is unknown, the reduction in protein synthesis resulting from asparaginase-induced depletion of

asparagine and glutamine has been implicated. This observation is indeed consistent with higher concentrations of plasma asparagine and glutamine compared to L-ASP. Appropriate information has been included in the SmPC in relation to known/potential AEs, which is consistent with similar MP and thus, acceptable.

3.3 Risk management plan

Safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Summary of safety concerns	
Important identified risks	Hyperglycemia Coagulation disorders Hepatic toxicity Pancreatic toxicity
Important potential risks	Transfusion accident Volume overload Co-administration with anticoagulants Co-administration with vaccines
Missing information	Use in patients with hepatic Impairment Use in patients with renal Impairment Use during pregnancy Use during breast-feeding

Conclusion

Still, there is a need for further revision of the List of Safety Specifications to make it consistent with similar medicinal products, even though the frequently of some AEs was less common than for L-asp.

Pharmacovigilance Plan

The Applicant proposes routine pharmacovigilance for the safety concerns in the RMP.

Due to the very limited study population and as a consequence the limited knowledge on safety, at least active surveillance should be in place. A Registry should be considered as additional pharmacovigilance activity to more proactively gain knowledge on the safety profile. This could be an existing disease registry (ALL registry), or a drug registry to further examine the safety profile of this orphan drug indicated for a specific condition.

Also, any ongoing clinical studies in the proposed indication that will obtain information on any of the safety concerns listed in the RMP should be included in the Pharmacovigilance Plan.

Risk minimisation measures for Graspa

The Applicant proposes routine risk minimisation for the safety concerns in the RMP.

The current view is that the proposed routine risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

Public summary of the RMP

The public summary of the RMP requires revision. The currently used language is not quite suitable for a lay audience.

PRAC Outcome

The PRAC adopted the proposed List of Questions in September 2016. The PRAC agreed that the risk management plan version 1.1 could be acceptable provided the applicant implements the changes to the RMP as stated in section 6.4 (LoQ).

The PRAC agreed that the MAH should explore the use of a registry as additional pharmacovigilance activity to more proactively gain knowledge on the safety profile, in view of the very limited study population and the limited knowledge on safety. This could be an existing disease registry (ALL registry), or a drug registry to further examine the safety profile of this orphan drug indicated for a specific condition.

Additionally the applicant should include any ongoing clinical studies in the proposed Indication that will obtain information on any of the safety concerns listed in the RMP in the Pharmacovigilance Plan. The MAH should also try and include sufficient number of patients in these ongoing studies.

The RMP should include a discussion of the measures in place to ensure product traceability.

3.5 Pharmacovigilance system

The Rapporteurs consider that the Pharmacovigilance system as described by the applicant fulfils the requirements and provide adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

4. Orphan medicinal products

ERY001 (active substance: L-asparaginase encapsulated in erythrocytes) has been granted three Orphan Drug Designation (ODD) in Europe: one for the treatment of Acute Lymphoblastic Leukaemia in October 26th, 2006: Decision EMEA/OD/033/06, one in the treatment of pancreatic Cancer May 15th, 2009: Decision EMEA/OD/100/08 and in the treatment of Acute Myeloid Leukaemia February 9th, 2013: Decision EMEA/OD/167/12.

According to the conclusion of the COMP the prevalence of the “condition” acute lymphoblastic leukaemia is approximately 0.5 in 10,000 persons in the European Union, which, at the time of designation, corresponded to about 23,000 persons in total.

5. Benefit risk assessment

Graspa is a personalized dispersion for infusion of Eryaspase (proposed INN), and is an L-asparaginase encapsulated in red blood cells and is manufactured from L-asparaginase derived from E. Coli. During the MA procedure, the Applicant proposes a major quality change in manufacturing consisting in using recombinant L-asp instead of native L-asp as the marketed enzyme to be encapsulated into erythrocytes.

The enzyme L-asparaginase has been used in the treatment of ALL since 1970. L-asparaginase results in depletion of serum asparagine, which in turn inhibits the protein synthesis, causes cell cycle arrest in the G1-phase and apoptosis in susceptible leukemic cells. L-asparaginase is an integral component of multi-agent chemotherapy regimens.

The initially claimed indication is: "*Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of paediatric and adult patients with acute lymphoblastic leukaemia, who have either relapsed or failed first line treatment. Graspa is also indicated for the treatment of paediatric and adult patients with acute lymphoblastic leukaemia and hypersensitivity to asparaginase*."

A revised wording is proposed during the procedure (D150): "*Graspa, in combination with multi-agent chemotherapeutic regimens, is indicated for the treatment of children over 1 year of age and adult patients with Philadelphia chromosome negative acute lymphoblastic leukaemia, who have either relapsed or failed first line treatment.* "

The evidence of the efficacy of Graspa for the claimed indication is provided by the results of the pivotal Phase II/III Study GraspaLL 2009-06. Two supportive studies are also presented (Study GraspaLL/ GRAALL SA2-2008 and Study GraspaLL 2005-01). In the three studies, main demonstration of efficacy relies on asparaginase activity parameters, while clinical endpoints are secondary objectives.

Benefits

Beneficial effects

Results from the pivotal Study 2009-06 show that in patients treated with Graspa with mild or without previous HS reactions, the mean duration of asparaginase activity >100 IU/L measured in whole blood was higher than in L-ASP arm, with a mean of 20.2(SD: 2.59) days and 12.25 (SD: 7.62) days, respectively. Statistically significant non-inferiority was established excluded the non-inferiority margin of 0.8; p value for non-inferiority <0.001), as well as superiority of Graspa over L-ASP (p <0.001). None of the patients (0/26) in the Graspa arm had hypersensitivity reactions related to study drug during induction, compared to 13 (46.4%) in the L-ASP arm, reaching statistically significant differences in proportions, -0.46 (95% CI for difference in proportions: (-0.69; -0.24)).

Consistent results were found across key subgroup analyses, namely children and adults, risk groups, high versus standard risk, and antibody status at baseline. Sensitivity analyses were based on cut-offs of 75 IU/L and 400 IU/L for asparaginase activity measured in whole blood during induction period and showed consistent results. Sensitivity analyses for the co-primary HS reactions, namely any hypersensitivity reactions regardless of relationship to study drug (11.5% Graspa vs 50% control, p<0.002), and all hypersensitivity reactions during the full 28-day period (including switching to another L-asparaginase formulation) (15.4% Graspa vs 53.6%control), supported the conclusion of the superiority of Graspa over L-ASP in terms of reducing the incidence of related hypersensitivity reactions.

Results for key secondary endpoints are not fully consistent, in particular data in the initially established PEP: the mean duration of asparagine depletion ≤ 2 μM was higher in the L-ASP arm compared to the Graspa arm, with a mean of 12.3 (SD: 7.8) days and 7.1 (SD: 5.7) days, respectively), thus the non-inferiority could not be demonstrated.

Uncertainty in the knowledge about the beneficial effects

Surrogate efficacy endpoint

Whilst it could be said that the aim of the study had been met and efficacy had been show, there are a number of issues which call the use of 'mean duration of asparaginase activity>100U/L in whole blood' as a surrogate measure of asparaginase efficacy in this setting into question. These include:

- Choice of control treatment regimen

The Applicant argues that this was the standard protocol used by the European Organization for Research and Treatment of Cancer - French Acute Lymphoblastic Leukaemia group, it is recognised that it may have been difficult to impose another protocol for this study. So, although the administration regimen of L-ASP is not considered optimal, it can be acceptable as it is used in clinical practice. However, apart from the COOPRALL protocol, it remains uncertain to what extent the results of the pivotal study can be reasonably generalised to other protocols used in clinical practice. Thus, the currently requested broad indication requires further justification.

Regarding the internal validity of the pivotal trial, the Applicant's response should be completed. The Applicant should specify the proportions of patients who received L-ASP via the IV and IM routes.

- Evaluation of asparaginase activity in whole blood

The results of the asparagine activity evaluation are expected do not reflect the activity of the product in its biologically active form (compartmentalised within erythrocytes) as erythrocyte lysis, releasing free asparaginase, is required to assay asparaginase in whole blood. Whilst the whole blood asparaginase activity peaks at 2133U/L, far higher than is required to achieve complete asparagine depletion instantaneously and much higher than the activity levels seen with the comparator, time to asparagine depletion was longer than that of the comparator and duration and incidence of asparagine depletion were notably shorter and smaller respectively. The PK data for Graspa are not compatible with the PD outcomes. This evidence suggests that the asparaginase activity of Graspa as measured in whole blood, vastly overstates the activity of the product in vivo. This also questions the clinical utility of this parameter based on old requirements to monitor treatment response and given that this is becoming common clinical practice due to the availability of other asparaginase formulations, further discussion is needed on the information that should be included in the SmPC in order to guide clinicians and ensure an adequate use.

Complete Remission

There are some issues with the evaluation of complete remission. Evaluation of CR should have included CSF evaluation and objective evaluation of extramedullary disease, which it didn't. Further the CR endpoint definition should have incorporated all features of both clinical and cytologic remission. It was also unclear whether those who received rescue treatments before the end of induction were included in the analyses. The CR data currently cannot be considered robust and it cannot be concluded that Graspa is associated with higher rates of complete remission.

In the updated CR analysis (ITT), the CR rates were 69% (Graspa) vs. 50% (L-ASP). However, although very limited, MRD data were in favour of L-ASP. The new requested sensitivity analysis based on exposure to study drug (rather than drug discontinuation) and using all CR data related to study drug is considered more reflective of the effects of study drug in the highest possible number of patients. CR and MRD after induction are considered the most reliable efficacy endpoint to support the

efficacy of Graspa compared to L-ASP. However, the evaluation of this key secondary endpoint is undermined by the fact that only CBC and bone marrow data have been properly documented while clinical criteria of CR are not.

Survival

Overall survival and event free survival analyses only 12 month follow up data were available and overall the data could not be considered mature. Updated results, OS at 36 M favours Graspa treated arm, but results are presented in an incomplete way. Updated EFs and relapse rates have not been provided. It is worth noting that differences in OS and EFS are not expected to be shown in this study, given the enrolled population, the treatment being compared and the large number of concomitant medicines used – hence the use of PK/PD surrogate efficacy endpoints in the investigation of differences in efficacy between two asparaginase products. Although due to the limited number of patients and switching, firm conclusions can hardly be drawn based on these clinical outcome parameters, a formal discussion on the robustness of these results is still awaited.

Key surrogate efficacy measure

There were clear clinically relevant differences in mean duration of plasma asparagine depletion and proportion of patients with at least one day of asparagine depletion between the two asparaginases non-allergic study arms (L-asparaginase > Graspa). These data showing a significantly greater effect of L-asparaginase than Graspa on asparagine clearance were in direct opposition to the other measures of efficacy in the development programme.

What is striking about the asparagine depletion data are the extent of the differences between study arms despite the existence of some bias within the study design against the L-asparaginase arm and in spite of the long-lasting asparaginase activity in Graspa treated arm.

It is clear that despite its activity in whole blood, Graspa's ability to hydrolyse plasma asparagine is significantly impaired as almost all the asparaginase is sequestered within erythrocytes and its activity is limited by the transport of asparagine across the erythrocyte membrane and less importantly lysis of erythrocytes. With regard to establishing the anti-leukaemic effect of asparaginases the critical factor is the capacity to deplete asparagine from the plasma, as this is the main source of asparagine for leukaemic cells, as it cannot be synthesized intracellularly. Therefore, despite the presence of asparaginase in large quantities in whole blood, Graspa is not considered to have a profound anti-leukaemic effect, due to the above limitations in hydrolysing asparagine.

Further to the discussion above, duration of asparagine depletion is considered to be the most appropriate and clinically relevant surrogate measure for efficacy of asparaginases. However, a differential effect on ex vivo asparagine depletion cannot be firmly ruled out given the lack of accurate methods to inhibit ex vivo degradation and to the existence of different asparaginase activity levels in plasma between treatment arms. The main problem being that the magnitude of the ex-vivo degradation in each treatment arm cannot be estimated reliably. So, at present it is uncertain to what extent differences in mean duration of asparagine depletion are due to true differences in ex-vivo degradation and/or to real differences in activity between treatment arms, making difficult drawing conclusions from these data.

Risks

Unfavourable effects

In the 3 key studies (pivotal Phase II/III and supportive studies), all patients experienced at least one AE; of these 80.4% had related AE. The majority of the events resolved, with 15.7% of the patients reported AEs leading to either temporary or permanent discontinuation of the study drug. The majority of the patients (89.2%) experienced at least one SAE, with 27.5% reporting SAEs that were considered study-drug related. The related SAEs with a fatal outcome accounted for 3.9%. In safety population of completed ALL studies, AE rate was similar in children and adults. However, adult population (N= 55) had a higher rate of events leading to study drug discontinuation (23.6% vs. 6.4%), related SAEs (41.8% vs. 10.6%), SAEs with fatal outcome (65.5% vs. 17.0%), and related SAEs with fatal outcome (7.3% vs. 0%).

The most commonly reported AEs were myelosuppression, impaired coagulation, hepatotoxicity, pancreatic toxicity, hyperglycemia, and drug hypersensitivity, infections/sepsis, which are common known AEs associated to L-asparaginase treatment in the ALL population.

Myelosuppression is consistent with the underlying disease and reported similarly in both treated arms of the pivotal study. The most common AEs were leukopenia, thrombocytopenia and neutropenia, which were reflective of the disease characteristics and treatment with chemotherapy.

Non-allergic patients: A lower incidence of known class effect AEs were reported for Graspa compared to L-asp in the pivotal study: impaired coagulation disorders included hypofibrinogenemia (30.8% vs. 67.9%), decreased anti-thrombin III (15.4% vs. 71.4%), prolonged activated partial thromboplastin time (PTT) (3.8% vs. 14.3%), and decreased prothrombin level (0% vs. 10.7%), in the Graspa and LASP arms, respectively; hypersensitivity reactions accounting for 19.2% and 64.3% of patients in Graspa and in L-ASP arm, respectively; elevated AST and/or ALT occurred in 57.7% and 42.9% of patients in the Graspa and L-ASP arms, respectively; hyperbilirubinemia and hepatotoxicity accounted for 7.7% and 11.5% in the Graspa arm, compared to 28.6% and 21.4% in L-ASP arm; a total of 34.6% and 57.1% had elevation of pancreatic enzymes in Graspa and L-ASP arms, respectively. Similar pattern was observed with hyperglycemia (15.4% and 28.6%) and hypertriglyceridemia (7.7% and 10.7%).

In the randomized treatment arms (Graspa and L-ASP) of the pivotal study, 89% of patients and 93% of patients reported SAE in Graspa and L-asp treated arms, respectively. The most common SAEs were febrile bone marrow aplasia (39% Graspa vs 25% L-asp), febrile neutropenia (15% Graspa vs 18% L-asp), and mucosal inflammation (11.5% Graspa vs 14.3% L-asp), and infection (including sepsis/septic shock (34.5% Graspa vs 13.3% L-asp). By contrary, disease progression (11.5% Graspa vs 14% L-asp) and drug HS reactions/other allergic reactions (11.5/7.7% vs 25/0% in Graspa and L-asp respectively) were most commonly reported in the L-asp treated arm.

In the pivotal study, there was a total of 14 fatal events during the study, 2 (7.7%), 6 (21.4%) and 6 (23.1%) with Graspa, L-ASP, and Graspa-s (allergic patient), respectively. Disease progression was the primary cause of death in the 3 arms, followed by AEs (infections). As expected, due to the correlation between asparaginase allergy and anti-drug antibody (ADA) positivity, at baseline, approximately half of patients in the asparaginase allergic arm were ADA positive. Interestingly, immunogenicity data in study GraspaLL 2009-06 did not suggest any differences between Graspa and L-asparaginase in the three study arms, in that rates of positive conversion were similar. However the data have to be placed into the context of those who were negative at baseline; i.e. those in whom it was possible to have positive conversion. Viewed from this perspective, conversion to positive antibody status occurred in 8

(30.8%) patients treated with Graspa, and in 7 (25.0%) patients treated with L-ASP. Median time to antibody positivity was not reached in both Graspa and L-ASP arms.

Uncertainty in the knowledge about the unfavourable effects

The main uncertainty is an immediate consequence of the limited safety database and the fact that L-asparaginase treatments are given as part of a multi chemotherapeutic regimen, thus, making difficult the assessment of potential differences between L-asp formulations. Nevertheless, it is recognised that based on data available the overall safety profile is within that known for already marketed asparaginase formulations, with the addition of transfusion reactions, which is the route of administration for Graspa.

Whilst in the Graspa arm AST and ALT, as adverse events occurred more frequently than in the L-asparaginase arm, hepatic events, defined as incidence of simultaneously elevated AST/ALT, bilirubin and/or evidence of hepatic failure, were not increased. It was further, it is reassuring that elevated GGT and hyperbilirubinaemia AEs occurred less frequently in the Graspa arm. The higher frequency of elevated AST/ALT reports may be due to concomitantly administered cytotoxic agents or could be a chance finding. The concern regarding a direct hepatotoxic effect through the removal of asparaginase laden erythrocytes from the circulation by the hepatic reticuloendothelial system is currently theoretical; but as the safety database is small, cannot be excluded.

Some subset of patients are not represented (or underrepresented) in the database presented: i.e patients >65 years, patients with chromosome Philadelphia +, patients with history of G4 HS reactions to L-asparaginase, history of pancreatitis or transfusion incidents. Appropriate information has been included in the SmPC and the RMP.

Given the significantly lower hypersensitivity rate in Graspa administered patients, it is notable that conversion rates were similar in both treatment arms. However, it should be noted that the total number of patients included in the direct comparison of the two asparaginase products is limited. Further (as noted in the PK section of the AR) very limited detail is available about the immunogenicity assay used in the development programme. The higher rate of serious HS reactions adds uncertainties on to what extent encapsulation truly prevents from immunological reactions. In relation with this issue, the applicant is aware that the assays used to assess antibody status are not optimal. Therefore, immunogenicity cannot be properly analysed. Consequently, one of the two goals of this clinical development (significantly less immunogenicity is expected with Graspa) is highly questioned at this time, although the rate of allergic reactions was so different for Graspa and L-ASP (in the pivotal trial, none of the patients in the Graspa arm had evidence of hypersensitivity reaction during induction compared to 46% of patients in the control arm, who experienced hypersensitivity reactions). Reliable data about immunogenicity should be provided. New analyses of the samples from the pivotal trial will be provided and timelines should be specified.

Effects Table

Table 2. Effects Table for Graspa in the treatment of relapsed ALL (data cut-off:28th August, 2014).

Effect	Short Description	Unit	Graspa	L-asp	Uncertainties / Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	Graspa	L-asp	Uncertainties / Strength of evidence	References
Asparaginase activity >100 IU/L	Mean duration in days, measured in whole blood	Mean days 95%CI	20. 2 (19, 21)	12.25 (7.0; 17)	Non-inferiority demonstrated Testing for superiority pre-planned and demonstrated. P<0.001	See Section 2.3.7 Overview
Incidence HS reactions	Related to Study drug during induction	%	0 (26.9%)	46.4 (71.4%)	OL design might have biased this assessment. Sensitivity analysis presented regardless of causality show consistent favourable results for Graspa treated arm	
Asparagine depletion <2µM	Asparagine depletion during induction (Mean)	Mean (days) 95% CI	7.1 (4.82; 9.44)	12.3 (9.28; 15.32)	Non inferiority not demonstrated based on this parameter. Unreliability of comparison due to unknown degree and potentially different ex-vivo degradation between study arms. Poor correlation with asparaginase activity levels in Graspa treated arm, which questions its validity	
CR	During induction	% (95%CI)	65.4%(51.6; 89.8)	39.3%(23. 3; 63.1)	High discontinuation rate and switching to other formulations might have contributed to these results to an unknown extent. Extramedularly disease not measured.	
MRD	as presence of leukemic cells < 10-3	%	44%	57%	Idem Non-significant diff	
RR	at 12 months		26.3%; 95% CI:(9.1; 51.2))	16.7%; 95% CI: (3.6 ; 41.4)	Idem. Updated results not presented for this outcome	
EFS/OS	at 12M	Median EFS (months) Median OS	NR NR	11.6 NR	Idem (Immature). EFS 23.1% vs 32.1% at 12 months Death rate at 36 months 35% vs 43% Graspa and L-asp, respectively Incomplete presentation of results at D150	
Unfavourable Effects						
Overall incidence of AE (SAE)	Completed ALL studies	%	100% (88.5%)	100% (92.9%)		
Fatal events		%	7.7 (23.1 Graspas)	21.4		
Myelosuppression	AE Leucopenia Thrombocytopenia Neutropenia (febrile Np Aplasia Sepsis/shock)	%	 88.5% 80.8% 76.9% (15.4%, 38.5%, 23%)	 89.3% 92.9% 82.1% (17.9%, 25%, 10.7%)		See Section 2.3.8 Overview AR
Coagulation disorders	AE (SAE)	%	46.2 (5.6)	89.3 (8.0%)		

Effect	Short Description	Unit	Graspa	L-asp	Uncertainties / Strength of evidence	References
Hepatotoxicity	AE (SAE)	%	73.1 (10.5%)	64.3 (5.6%)		
Pancreatic events	AE (SAE)	%	34.6% (11.1%)	57.1% (25%)		
Diabetogenic events	AE (SAE)	%	23.1 (0%)	39.3 (0%)		
Transfusion events	AE (SAE) Entire safety population	%	4.9% (1.1%)	NA		
ADA convertion		%	30.8% (26.9% Graspas)	25%		

Balance

Importance of favourable and unfavourable effects

There are major concerns related to the demonstration of efficacy of this asparaginase formulation. The claimed superiority, and even the non-inferiority, over native L-asparaginase in terms of whole blood asparaginase activity is not substantiated by consistent results in duration of asparagine depletion, which is considered the primary surrogate measure of asparaginase efficacy. Duration of asparaginase activity >100UI within whole blood is clearly NOT a good estimator of the efficacy for the new asparaginase formulation given that it considerably overestimates the activity of Graspa in its biologically active form (i.e. asparaginase compartmentalized within erythrocytes). Despite the identified bias in favour of Graspa within the study design, E. Coli L- asparaginase was associated with significantly longer and more frequent asparagine depletion than Graspa. So, at present the validity of the comparison based on PD parameters is seriously questioned and thus, the non-inferiority in the activity of Graspa vs available L-asparaginase formulations remains to be established.

Since PD markers are not helpful at present to adequately predict the efficacy of Graspa, this application mainly relies on clinical outcomes. Due to the limited sample size of the pivotal trial, the estimates of efficacy are notably uncertain and non-inferiority of Graspa vs L-ASP cannot be established. This remains currently a major concern.

Graspa seems to be better tolerated compared to the native formulation based on the lower incidence of HS reactions and some key AEs known to be associated with L-asparaginase treatment. This is considered an added value over the native formulation, due to the high rate of treatment discontinuations and limitations to subsequent treatments. However, uncertainties remain on the immunogenicity assessment of Graspa since the Applicant has admitted that the ADA assays have not been validated.

Benefit-risk balance

The benefit-risk balance is considered to be negative

Additional considerations on the benefit-risk balance

L-asparaginase is an integral component of multi-agent chemotherapy regimens used in induction of remission in pediatric and adults, which are given over a treatment period of several months for patients with ALL. Therefore, it would be unrealistic to mandate the normal clinical endpoints (CR, MRD rate, PFS and OS) when investigating new L-asparaginase products. Thus, as already recognised by CHMP, the level of L-asparagine depletion, the duration of the depletion or the level of asparaginase activity in plasma have been proposed as surrogate PK endpoints given the strong correlation with clinical endpoints demonstrated so far.

The efficacy component of the co-primary endpoint initially selected was asparagine depletion, but due to differential ex-vivo degradation in Graspa and L-asp, the reliability of the comparison was considered limited and thus, the Company, in agreement with CHMP, proposed a new co-primary endpoint based on duration of asparaginase activity >100IU/L and rate of HS reactions due to study drug. Formally, the non-inferiority and the superiority of Graspa in terms of duration of asparaginase activity over 100 IU/L has been demonstrated, as well as the superiority in the incidence of HS reactions related to Graspa.

However, due to the inconsistent results observed between asparaginase activity in whole blood (mean 20.2 days) and asparagine depletion (mean 7.1 days) in the Graspa treated arm, and the high rate of treatment discontinuations due to not reaching the target of depletion, it is seriously questioned that the duration of asparagine activity >100IU/L is a good estimator of the efficacy of this asparaginase formulation. In fact, asparaginase activity in plasma in considered to correlate with asparagine depletion, which is the ultimate goal of treatment, as shown for other asparagine formulations. This has been shown for the L-asp treated arm in the pivotal study (12.25 days mean duration of asparagine activity >100IU/L vs 12.3 days mean duration of asparagine depletion). However, this correlation does not exist for Graspa, for which duration of asparaginase activity seems to overestimate the true effect.

There are major concerns related to the demonstration of efficacy of this asparaginase formulation and to what extent this may be counterbalanced by the apparent better tolerability. Asparagine depletion results might be biased to an unknown extent in favor of L-asp, whilst asparaginase activity (in whole blood for Graspa vs plasma for L-asp) appears to overestimate the treatment effect in favor of Graspa in a substantial extent. Therefore, no reliable comparisons can be made based on PD markers.

Results in clinical endpoints are of limited value to alleviate the concerns, given the many factors which may be contributing to these results, including the limited sample size, the open label study design and switching to a new asparaginase formulation, which might have been high in the pivotal study.

Overall, if the correlation between PD results and treatment effect is not considered plausible, conventional clinical endpoints would be needed to demonstrate the efficacy of Graspa in comparison to currently available asparaginase formulations. Due to the limited sample size of the pivotal trial, the estimates of efficacy are notably uncertain and non-inferiority of Graspa vs L-ASP cannot be established. This remains currently a major concern. In the updated CR analysis (ITT), the CR rates were 69% (Graspa) vs. 50% (L-ASP). However, although very limited, MRD data were in favour of L-ASP. Additional sensitivity analysis based on exposure to study drug (rather than drug discontinuation) and using all CR data related to study drug have been requested and are considered more reflective of the effects of study drug in the highest possible number of patients. CR and MRD after induction are considered the most reliable efficacy endpoint to support the efficacy of Graspa compared to L-ASP. However, the evaluation of this key secondary endpoint is undermined by the fact that only CBC and bone marrow data have been properly documented while clinical criteria of CR are not.

There appears to be significant inherent bias in favour of Graspa within the COOPRALL protocol - shorter period of L-asparaginase administration, presence of an extended asparaginase dosing interval between F1 and F2, use of a non-PEGylated asparaginase as a comparator and use of a lower than recommended daily dose for L-asparaginase.The Applicant argues that this was the standard protocol used by the European Organization for Research and Treatment of Cancer - French Acute Lymphoblastic Leukaemia group. So, although the administration regimen of L-ASP is not considered optimal, it can be acceptable as it is used in clinical practice. However, apart from the COOPRALL protocol, it remains uncertain to what extent the results of the pivotal study can be reasonably generalised to other protocols used in clinical practice. Thus, the currently requested broad indication requires further justification (MO).

Graspa seems better tolerated compared to the native formulation based on the lower incidence of most well-known toxicities of asparaginase with Graspa than with L-ASP: hypersensitivity reactions (as expected since asparaginase is mostly encapsulated) but also abnormal pancreatic enzymes, coagulation factors and parameters, liver enzymes (except for transaminases), hypoalbuminaemia, hyperglycaemia, hepatotoxicty, pancreatitis, thrombotic/haemorrhagic events. This is considered an added value over the native formulation, due to the high rate of treatment discontinuations and limitations to subsequent treatments. However, there are some uncertainties related to the immunological evaluation in the present dossier, which need to be solved. Currently the efficacy of Graspa, through PK and PD surrogates, has not been shown to be non-inferior to the L-asparaginase comparator. Therefore, the newly proposed indication, despite being restricted to the second line treatment and without any additional claim for patients with HS reactions, cannot be accepted at present. In the absence of reliable PD parameters, further discussion is needed on the robustness of the results in conventional clinical endpoints of the pivotal study in order to demonstrate the therapeutic efficacy of Graspa. In addition, ADA data with a validated assay are required prior to any MA

In addition, quality Major Objections have been raised as regards the control strategy and process validation, and the little information provided on the manufacture of E. coli L-asparaginase that is used for the encapsulation in erythrocytes to obtain eryaspase. The comparability exercise to be extended and the need for additional bridging studies discussed.

An additional issue is that the CHMP disagrees with the Applicant´s definition of drug substance for this medicinal product as being eryaspase. Instead, and in line with previous advice provided by the CHMP and CAT to the Applicant, the asparaginase enzyme, either native L-asparaginase or the newly proposed recombinant asparaginase, has to be considered as the Drug Substance. This has been confirmed by the BWP. Thus, the CHMP considers that the active substance eryaspase contained in the medicinal product Graspa is not to be qualified as a new active substance in itself.

Conclusions

The overall B/R of Graspa is negative.