20 July 2023
EMA/429470/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Enrylaze

International non-proprietary name: crisantaspase

Procedure No. EMEA/H/C/005917/0000

Note

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
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List of abbreviations

ADA	Antidrug Antibody(ies)
AIC	Akaike Information Criterion
ALL	Acute Lymphoblastic Leukaemia
BLQ	Below Limit Of Quantification
BSA	Body Surface Area
BSV	Between-Subject Variability
BUN	Blood Urea Nitrogen
CCIT	Container Closure Integrity Testing
CCP	Confirmatory Cut Point
CCS	Container Closure System
cGMP	Current Good Manufacturing Practice
CHMP	Committee For Evaluation Of Human Medicinal Products
CI	Confidence Interval
CL	Clearance
CL/F	Clearance
CPP	Critical Process Parameter
CPV	Continued Process Verification
CQA	Critical Quality Attribute
CSR	Clinical Study Report
CV	Coefficient Of Variation
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia Coli*
EMA	European Medicines Agency
EoP	End of Production Bank
E-R	Exposure-Response
EU	Endotoxin Unit
FDA	Food And Drug Administration
FOCEI	First Order Conditional Estimation With Interaction
HCP	Host Cell Protein
HPC	High Positive Control
ICH	International Council For Harmonization Of Technical Requirements For Pharma For Human Use
IIV	Individual Variability
IM	Intramuscular
IPC	In-Process Control
IPM	In-Process Monitoring
ISI	Integrated Summary Of Immunogenicity
ISR	Incurred Sample Reanalysis
IV	Intravenous
IVIM	Intravenous-Intramuscular
Jazz	Jazz Pharmaceuticals

JZP-458	Recombinant Crisantaspase Produced in *Pseudomonas Fluorescens*; RC-P (Also R As JZP458)
kDa	Kilodalton
KPP	Key Process Parameter
LBL	Lymphoblastic Lymphoma
LLOQ	Lower Limit Of Quantification
LPL	Low Positive Control
MCB	Master Cell Bank
MD	Multiple Dose
MRD	Minimum Required Dilution
MWF	Monday, Wednesday, Friday
NA	Not Applicable
NAb	Neutralising Antibody
NC	Negative Control
NCPP	Non-Critical Process Parameter
NF	National Formulary
NHANES	[Centers For Disease Control] National Health and Nutrition Examination Survey
NSAA	Nadir Serum Asparaginase Activity
OFV	Objective Function Value
OOS	Out Of Specification
PC	Positive Control
PC	Process Characterisation
pCCP	Potential Critical Process Parameter
pcVPC	Prediction-Corrected Visual Predictive Check
PD	Pharmacodynamic
Ph. Eur.	European Pharmacopoeia
PIP	Paediatric Investigation Plan
pI	Isoelectric Point
PK	Pharmacokinetic(s)
PopPK	Population Pharmacokinetic(s)
PP	Polypropylene
PP	Process Parameter
PPQ	Process Performance Qualification
PRS	Primary Reference Standard
PS80	Polysorbate 80
PSCP	Plate-Specific Screening Cut Point
PV	Process Validation
Q1	First Quartile
Q2	Second Quartile
Q48h	Every 48 Hours
QOD	Every Other Day
QbD	Quality By Design

QC	Quality Control
QTTP	Quality Target Product Profile
RCB	Research Cell Bank
RC-P	Recombinant Crisantaspase Produced in *Pseudomonas Fluorescens* (JZP-458; Als To As JZP458)
RH	Relative Humidity
RS	Reference Standard
SAA	Serum Asparaginase Activity
SAC	Serum Asparaginase Concentration
SmPC	Summary Of Product Characteristics
t½	Terminal Elimination Half-Life
T-ALL	T-Cell Acute Lymphoblastic Leukaemia
TDM	Therapeutic Drug Monitoring
V	Volume Of Distribution
Tmax	Time Of Cmax
TSE	Transmissible Spongiform Encephalopathies
UF/DF	Ultrafiltration/Diafiltration
USP	United States Pharmacopoeia
WCB	Working Cell Bank
WRS	Working Reference Standard

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Jazz Pharmaceuticals Ireland Limited submitted on 24 May 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Enrylaze, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

Enrylaze is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) in adult and paediatric patients who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase.

Enrylaze is indicated in patients 1 month of age and older.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0453/2021 on the agreement of a paediatric investigation plan (PIP).

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.5. Scientific advice

The applicant received the following scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
27 February 2020	EMA/CHMP/SAWP/81985/2020	Ewa Balkowiec-Iskra, Pierre Demolis and Serena Marchetti
16 March 2021	EMA/SA/0000055593	Ewa Balkowiec-Iskra, Pierre Demolis

		and Serena Marchetti

The scientific advice pertained to the following quality, non-clinical and clinical aspects:

- The suitability of the analytical comparability and process development strategy to support a future Article 8(3) full-mixed MAA;
- Whether, based on the totality of the data available for asparaginases, the nonclinical strategy supports the development of JZP-458 and an Article 8(3) full-mixed MAA;
- The adequacy of Part A of the Phase 2/3 study protocol (Study JZP458-201), to demonstrate the efficacy and safety of Crisantaspase for MAA;
- The adequacy of Part B of the Phase 2/3 study protocol (Study JZP458-201) to determine an appropriate IV dose and schedule of Crisantaspase;
- Whether the Phase 2/3 study data, together with the quality and nonclinical development programme, are sufficient to establish the benefit/risk of JZP-458 as a component of a multiagent chemotherapeutic regimen for the treatment of patients with ALL/LBL who have developed hypersensitivity to Escherichia coli (E. coli)-derived asparaginase.

1.6. Steps taken for the assessment of the product

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

Rapporteur: Paula Boudewina van Hennik (later replaced by Johann Lodewijk Hillege)

Co-Rapporteur: Simona Badoi

The application was received by the EMA on	24 May 2022
The procedure started on	16 June 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	6 September 2022
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	20 September 2022
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	19 September 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	29 September 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	13 October 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	24 March 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	2 May 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 May 2023
The CHMP agreed on a list of outstanding issues in writing to be sent to	25 May 2023

the applicant on	
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 June 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	5 July 2023
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Enrylaze on	20 July 2023
The CHMP adopted a report on similarity of name of the medicinal product with Iclusig, Blincyto, Besponsa, Kymriah and Tecartus on (see Appendix on similarity)	20 July 2023

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The proposed target indication for this marketing authorisation is:

'Enrylaze is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) in adult and paediatric patients who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase.

Enrylaze is indicated in patients 1 month of age and older.'

Acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) are a group of rare malignancies arising from precursor B cells (B-ALL/B-LBL), T cells (T-ALL/T-LBL), or both B and T cells (mixed lineage ALL/LBL).

Acute lymphoblastic leukaemia occurs upon a malignant transformation and proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. Lymphoblastic lymphoma is a rare, fast-growing, aggressive subtype of NHL, most often seen in teenagers and young adults. Lymphoblastic lymphoma is a cancer of immature lymphoblasts.

The 2008 and 2016 WHO classifications of lymphoid malignancies define B-ALL/B-LBL and T-ALL/T-LBL as single disease entities, categorised as leukaemia if there is extensive blood and marrow involvement, lymphoma if the process is confined to a mass lesion with no or minimal blood/marrow involvement, and leukaemia/lymphoma if both are present. ALL and LBL are similar in appearance and behaviour and both require intensive treatment.

2.1.2. Epidemiology

Acute lymphoblastic leukaemia/lymphoma (ALL/LBL) is the most common form of cancer in children, comprising approximately 30 percent of all childhood malignancies. The estimated global incidence of

ALL is 5.91 new cases per 100,000 or 153,315 cases in 2019. In Europe, the overall incidence of ALL is 11 per million per year, but the incidence peaks in childhood between 2 and 5 years of age (53 per million) and again after 50 years of age. Specifically, the median age at diagnosis for ALL is 15 years, with 55.4% of patients diagnosed at < 20 years of age. Approximately 12.3% of patients are diagnosed at ≥ 65 years. Lymphoblastic lymphoma is most often seen in teenagers and young adults.

2.1.3. Biologic features

The vast majority of cases of ALL/LBL have no known cause, but an increased incidence has been associated with certain environmental or genetic risk factors. These include age, exposure to chemotherapy or radiation therapy, and genetic disorders, including Down's syndrome. Approximately 75% of patients with B-ALL have recurrent chromosomal translocations or somatic aneuploidy, many of which have prognostic value.

2.1.4. Clinical presentation, diagnosis and prognosis

Clinical manifestations at presentation include constitutional symptoms (fevers, night sweats, weight loss), easy bruising or bleeding, dyspnoea, dizziness, and infection. Symptoms of haematological insufficiency, such as anaemia, granulocytopenia or thrombocytopenia, are common. More children than adults present with extramedullary disease, specifically in CNS and testis. Less than 10% of paediatric patients have symptomatic CNS involvement, although the frequency is higher in patients with mature B-ALL.

Survival rates for ALL/LBL in children have improved dramatically since the 1980s, with current five-year overall survival rates >85 percent. Five-year event-free survival rates are >93 percent for low-risk groups. Five-year OS decreases by age from 60% for young adults to 20% for adults over 65 years.

2.1.5. Management

In the EU, the majority of patients diagnosed with ALL and LBL follow a standard treatment protocol in which *E. coli*-derived L-asparaginase is an important component of first line therapy.

There are currently three preparations of asparaginase for use in patients with ALL/LBL:

- Short-acting *E. coli* derived L-asparaginase
- Long-acting pegylated (PEG)-asparginase*, E. coli* derived
- Short-acting *Erwinia chrysanthemi* L-asparaginase (crisantaspase) indicated for patients who develop a hypersensitivity reaction to *E. coli*-derived asparaginases

All three asparaginases share the same mechanism of action, the depletion of asparagine, yet differ in their pharmacokinetics. These differences have implications regarding the appropriate dose, schedule, and the interpretation of asparaginase activity measurements.

Because of their bacterial origin, L-asparaginases are allergenic and immunogenic. The most common toxicity of *E. coli*-derived L-asparaginases is hypersensitivity, which can manifest as allergic reactions or as "silent inactivation." Clinically evident allergic reactions are reported in up to one-third of patients receiving intensive schedules of the native forms of the enzyme and less frequently in patients receiving the pegylated form of asparaginase.

Switching patients who develop clinical hypersensitivity or silent inactivation to *E. coli*-derived asparaginase to alternative non-*E. coli*-derived preparation (e.g. Erwinase) can maintain survival benefits when adequate asparagine depletion is achieved. However, since 2016, there has been a worldwide shortage of Erwinase due to ongoing manufacturing issues.

While the absolute number of patients with hypersensitivity or silent inactivation to an *E. coli*-derived asparaginase is likely decreasing due to the predominant use of PEG asparaginase in frontline treatment and knowing that hypersensitivity rates to PEG asparaginase is lower than reported for native *E. coli* asparaginase, an unmet need remains for a reliable immunologically distinct L-asparaginase product that will allow patients to continue and complete prescribed treatment.

2.2. About the product

Enrylaze (also referred to in this report as JZP-458 or RC-P) was developed to overcome the shortage of the native *Erwinia* asparaginase crisantaspase, and is a new recombinant form of crisantaspase produced by Pseudomonas fluorescens. The applicant claims that the structure, stability and activity of Enrylaze are highly similar to native *Erwinia* asparaginase.

Asparaginase *Erwinia* chrysanthemi (recombinant) is an enzyme that catalyses the conversion of the amino acid L-asparagine into L-aspartic acid and ammonia. The mechanism of action is based on the inability of leukaemic cells to synthesise asparagine due to lack of asparagine synthetase activity, resulting in cytotoxicity specific for leukaemic cells that rely on exogenous asparagine for their protein metabolism and survival.

2.3. Quality aspects

1.1.1. Introduction

The finished product is presented as a sterile, single-use, preservative-free, aqueous solution for intramuscular (IM) or intravenous (IV) administration containing 20 mg of crisantaspase per mL (10 mg per vial) as active substance.

Other ingredients are: trehalose dihydrate, sodium chloride, sodium hydroxide, disodium phosphate, sodium dihydrogen phosphate monohydrate, polysorbate 80 and water for injection.

The product is available in a 2 mL Type 1 clear borosilicate glass vial, sealed with a halobutyl rubber stopper and aluminium overseal, and a violet plastic cap.

1.1.2. Active substance

1.1.2.1. General Information

The active substance is an asparaginase *Erwinia chrysanthemi* produced by recombinant DNA technology in *Pseudomonas fluorescens* and developed by Jazz Pharmaceuticals. The INN of the active substance is crisantaspase, but is also referred to as JZP-458 or RC-P. The crisantaspase structure is a non-disulfide bonded, tetrameric protein consisting of 4 identical subunits with a combined molecular weight of 140 kDa.

The crisantaspase amino acid sequence is published in the Swiss Prot database (reference number P06608) and is given below in Figure 1. Post-translational modifications are almost absent, with only low levels of deamidation, oxidation and succinimide formation detected.

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     10             20             30             40
22                                   ADKLPNIVI    LATGGTIAGS    AATGTQTTGY    50
51   KAGALGVDTL     INAVPEVKKL     ANVKGEQFSN     MASENMTGDV     VLKLSQRVNE    100
101  LLARDDVDGV     VITHGTDTVE     ESAYFLHLTV     KSDKPVVFVA     AMRPATAISA    150
151  DGPMNLLEAV     RVAGDKQSRG     RGVMVVLNDR     IGSARYITKT     NASTLDTFKA    200
201  NEEGYLGVII     GNRIYYQNRI     DKLHTTRSVF     DVRGLTSLPK     VDILYGYQDD    250
251  PEYLYDAAIQ     HGVKGIVYAG     MGAGSVSVRG     IAGMRKAMEK     GVVVIRSTRT    300
301  GNGIVPPDEE     LPGLVSDSLN     PAHARILLML     ALTRTSDPKV     IQEYFHTY      348
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Figure 1: Amino acid sequence of crisantaspase

Physicochemical, biological and immunochemical properties of the active substance are provided in Table 1 below. The general information is considered sufficient.

Table 1. General properties of crisantaspase

Property	Description
Description	Colorless to slightly yellow solution, clear to opalescent liquid
Activity	550-850 units[1] per mg protein
pH	7.0 ± 0.5
Molecular weight	140 kDa Tetramer. (Each individual subunit has a molecular weight of35 kDa)
Concentration	18.0-22.0 mg/mL

[1] The definition of a Unit of enzymatic activity is the amount (mg) of enzyme that catalyses the conversion of L-asparaginase (equivalent to 1µmol of ammonia produced) per reaction minute.

1.1.2.2. Manufacture, process controls and characterisation

The active substance is manufactured, tested and released at the following facilities, in accordance with Good Manufacturing Practice (GMP). The site responsible for the manufacture of crisantaspase is AGC Biologics A/S (legal name CMC Biologics, Inc.), Vandtaarnsvej 83B Soeborg, Copenhagen DK-2860, Denmark.

Description of the manufacturing process and process controls

The active substance manufacturing process has been adequately described.

Crisantaspase is produced as an intracellular product in a *Pseudomonas fluorescens* host. The upstream production process is started from a single cGMP working cell bank (WCB) vial and expanded in shake flasks. The shake flasks are incubated until a target cell density is achieved to be used as inoculum for the bioreactor operated in batch mode. A chemically defined medium is used in the production reactor. Expression of the plasmid gene encoding for the crisantaspase active substance is achieved through induction. Following induction, the culture is incubated until a target cell density is

achieved. The cells are then harvested by centrifugation and resuspended resulting in a wet cell paste that is prepared for downstream purification.

The harvested cell paste is mechanically lysed through a homogeniser to release the product. The homogenised cells are clarified by centrifugation and depth filtration prior to a series of chromatography steps to purify the product. An ultrafiltration and diafiltration (UF/DF) step is performed to concentrate and formulate the product in a buffer at pH 7.0. Final excipients are added to formulate the active substance in the final formulation step.

The maximum hold times at ambient conditions (17-25°C) for active substance intermediates have been justified by a stability assessment. Microbial control limits are in place for the upstream process and downstream in-process intermediates are tested (pre-filtration) for bioburden and bacterial endotoxins. Chromatography resins used for the chromatography steps to purify the product are each qualified for multiple cycles, while all membranes and filters are single use. Process parameter (PP) ranges and in-process controls (IPCs), along with acceptance criteria, are defined and justified as part of the overall control strategy.

A lot of crisantaspase active substance is derived from a single WCB vial, a single production reactor and a single downstream purification. The active substance batch scale is defined by the batch fermentation step. Sufficient details on the container closure system (CCS), including materials, dimensions and technical drawings are provided in the dossier. The CCS is gamma irradiated for sterility by the vendor and the vendor also confirms the seal integrity. No additional Quality Control (QC) testing is performed on the bottles. This is acceptable. Compliance of the materials with the Transmissible Spongiform Encephalopathy (TSE) note for guidance has been provided. Extractables and leachables studies concerning the active substance manufacturing process and have identified two substances of interest for which the levels are well below the acceptable limits.

Overall, the active substance manufacturing process is considered acceptable.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Raw materials used are either of compendial grade or controlled to ensure the quality and safety of the active substance, and to maintain the consistency of the manufacturing process. The materials are animal and human component free. Acceptable documents have been provided for raw materials of biological origin used in the establishment of cell substrate.

The recombinant crisantaspase expression strain was generated by transformation by electroporation of the expression plasmid into a *Pseudomonas fluorescens* host cell.

A research cell bank (RCB) of the cell line was created. The master cell bank (MCB) and WCB were manufactured in accordance with GMP. No materials of animal or human origin were used in the preparation of the RCB, MCB or WCB. All batches manufactured to date have used the WCB. Cell bank testing is performed according to the relevant ICH guidelines. An End of Production (EoP) bank was created from a GMP fermentation at the intended commercial scale and tests confirmed the plasmid to be fully retained at the end of production, with no structural or sequence modifications detected. The MCB will be monitored every 5 years for cell growth and viability, while monitoring of the WCB stability includes crisantaspase production and will be performed annually. A protocol for the generation and qualification of future WCBs has been presented and found adequate.

In conclusion, sufficient information is provided regarding testing of MCB and WCB and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

Control of critical steps and intermediates

Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Excursions from a process parameter (PP), normal operating ranges (NORs) or in-process test limits is managed through the deviation programme. No rejection limits are defined. The process steps for which critical process parameters (CPPs) are identified are defined as critical unit operations. A critical unit operation is considered to be any operation that has been determined to have a potential impact on one or more critical quality attributes (CQAs). NORs are defined for CPPs, but also for all key and non-critical process parameters (KPPs and NCPPs), and limits are defined for in-process controls (IPCs) and in-process monitoring (IPMs - attributes that have been evaluated to give supportive information about process performance). It is noted that CQAs are controlled by analytical methods at active substance level and viral safety is not of any concern given that the cell line is not of human or animal origin.

The overall control strategy is generally considered adequate for control of the crisantaspase manufacturing process.

Process validation

The process validation (PV) strategy for the crisantaspase manufacturing process encompasses three stages: process design, process performance qualification (PPQ) and continued process verification (CPV). During process design, supportive validation studies were performed and process relationships critical to the CQAs of the active substance and finished product were identified. The investigated CQAs include parameters for purity, product-related substances, product- and process-related impurities, and formulation related quality attributes. The available knowledge for each attribute was compiled and then each attribute was assigned as critical or non-critical. The set of supportive validation studies includes, but is not limited to, studies with regard to: cleaning and sterilisation, resin life time, shipping, in-process hold, process solutions and media hold, mixing and impurity clearance. The information presented is sufficient and considered acceptable.

The PPQ campaign consisted of full-scale active substance batches manufactured at set-point conditions. The PPQ protocols included prospectively defined acceptance criteria consisting of numerical limits for process parameters and process controls. Any excursions from the pre-defined NORs were evaluated to have no impact on product quality. All PPQ lots met the proposed commercial active substance release specification acceptance criteria and demonstrated consistent performance of the manufacturing process. During PV, process parameters that were designated as potential CPPs (pCPPs) were given a final criticality designation. A selection of raw material parameters and process parameters will be monitored for CPV.

In conclusion, sufficient information on process validation has been presented and the active substance manufacturing process is considered adequately validated, demonstrating that it can consistently produce crisantaspase of reproducible quality that complies with the predetermined specification and in-process acceptance criteria.

Manufacturing process development

The active substance manufacturing process was initially developed using small-scale runs. The process was transferred to AGC Biologics and scaled up, while processing steps remained the same. Analytical comparability between material manufactured by the small-scale and full-scale processes has been demonstrated. The full-scale process at AGC Biologics was used to manufacture the active substance for non-clinical and clinical use and is the intended commercial process. No major changes to unit operations were implemented throughout the development of the manufacturing process at AGC Biologics.

A bridging analytical comparability study was also performed to compare Enrylaze and Erwinase (a non-centrally authorised product containing the same active substance) in order to justify leveraging bibliographic literature on Erwinase for the replacement of own non-clinical and clinical study results. Comparable results were obtained for both materials.

Characterisation

Characterisation of crisantaspase active substance was performed to provide a comprehensive understanding of the biochemical, biophysical and biological properties of the protein, allowing a precise description of its quality attributes. State-of-the-art methods were used to evaluate the properties that relate to the molecular size distribution, post-translational modifications, hydrophobic heterogeneity, charge heterogeneity, molecular primary, secondary, tertiary and higher order structures, enzyme activity and kinetics, and thermal stability. The biological activity of crisantaspase is directly related to its enzymatic activity and ability to hydrolyze L-Asparagine. The analytical results are consistent with the proposed structure.

Furthermore, heterogeneity of the active substance was adequately characterised as well. Product-related variants, i.e. structural variants of the desired product, were isolated and identified as product-related substances or product-related impurities, based on their similarity to the main species in terms of their structure, size, hydrophobic properties, conformation and potency. Process-related impurities derived from or introduced during the manufacturing process were identified and evaluated for their toxicity risk with consideration of the maximum dose.

In summary, the characterisation is considered appropriate for this type of molecule.

1.1.2.3. Specification

Identity, quality and purity of each batch of crisantaspase active substance are assessed and confirmed according to the proposed specification provided in the dossier. The following tests are included in the active substance specification: general tests, identity, protein concentration, purity, potency and safety parameters.

Tests were selected based on a detailed understanding of the CQAs for crisantaspase active substance. The active substance specification does not include tests for several process-related and product-related impurities, for which the applicant provided an adequate justification. This approach is endorsed.

During the assessment, tightening of acceptance criteria for several quality attributes (i.e. colour, osmolality, purity, specific activity, bacterial endotoxins and residual HCP) and introduction of a stressed sample test (see Stability section below) were requested. The panel of tests is considered approvable, however the applicant was asked to commit to re-evaluate the requirement to conduct the stressed sample test as a routine test for active substance release when data from a sufficient number of batches are available **(Recommendation)**.

The specification was established based on regulatory guidelines, analytical capability, process capability and experience. A sufficient number of full-scale batches have been taken into consideration for specification setting for the active substance and justification. All batches were made with the same manufacturing process, to the same scale, including clinical and PPQ batches. Where possible, data sets have been supplemented with small-scale batches for statistical assessment.

Analytical methods

The analytical methods used have been adequately described. Method descriptions for all non-compendial analytical procedures are provided and validations are performed according to ICH Q2(R1).

The compendial methods have been verified to demonstrate the suitability for the intended purpose. Biological activity is analysed by assays that monitor the conversion of asparagine to aspartic acid and ammonia. To assure method validity and to comply with GMP requirements with respect to trending, the applicant was asked to commit to include additional controls for the test method for determination of catalytic efficiency **(Recommendation)**.

The applicant was also asked to commit to perform a supplemental validation of the analytical method for Protein Concentration, as well as to perform an evaluation of a potential potency assay method optimisation **(Recommendations)**.

The applicant was further asked to commit to develop a new HCP test method or, alternatively, to revalidate the current HCP method as recommended in Ph. Eur. 2.6.34 **(Recommendation)**.

Batch analysis

Batch analysis data (including PPQ batches and development batches) of the active substance were provided. All batch analysis data were in line with the acceptance criteria that applied at the time of testing. The results for batch release demonstrate a high level of batch-to-batch consistency.

Reference materials

The crisantaspase reference standards (RSs) follow a two-tiered approach with a primary RS and a working RS (WRS), where the PRS is used to (re)qualify (new) WRSs. The RSs are adequately qualified and considered representative of the commercial product. WRSs are requalified annually, while PRSs will be qualified annually for three years and every two years thereafter. Protocols for (re)qualification are provided. The information is considered acceptable.

1.1.2.4. Stability

The proposed shelf-life for the active substance is 36 months at -20°C ± 5°C. Stability studies are being conducted on all full-scale active substance batches manufactured to date, in accordance with ICH stability guidelines. The shelf-life specifications are identical with release specification, with the exception of: identity, potency, bioburden, bacterial endotoxins, residual HCP and residual DNA, which are tested only at release. The stability studies are performed using containers from the same material as the active substance CCS, although it is from a different supplier, which is acceptable. At the proposed long-term storage conditions (-20°C ± 5°C), the stability-indicating parameters showed no significant effect for up to 36 months. In addition, both active substance and finished product are identical in terms of concentration and formulation. The long-term finished product storage temperature of 2-8°C represents an accelerated temperature for the active substance material, for which 48 months of data are available for one finished product batch and 36 months of data are available for two additional finished product batches, which is considered supportive of the long-term stability of the active substance.

Results from accelerated (5°C ± 3°C for 9 months) and stressed (25°C ± 5°C/60% RH ± 5% RH for 3 months) conditions demonstrate degradation of the active substance, as can be expected, although acceptance criteria are still met. Freeze-thaw cycles are not considered to have a significant effect on the stability of the active substance.

During the active substance and finished product stability studies, for one batch an abnormal degradation was observed when the material was stored under accelerated and stressed conditions. This batch was not used for shelf life setting. To mitigate any risk, a routine stressed sample test has been implemented, with an adequate acceptance criterion, for the release of active substance batches and non-compliance with this release test will result in batch rejection. Forced degradation studies

have demonstrated that the crisantaspase active substance is resistant to high pH and light exposure, but is significantly degraded under oxidation and low pH conditions. The studies identify purity and specific activity as stability indicating analytical methods.

The provided stability data are considered supportive and the proposed shelf-life of 36 months at -20°C ± 5°C in the declared CCS is endorsed.

Commitments to complete the currently ongoing stability studies and to perform post-approval annual stability studies are provided. The proposed protocol (including adequate handling of any confirmed out of specification, OOS) is acceptable.

1.1.3. Finished Medicinal Product

1.1.3.1. Description of the product and pharmaceutical development

The crisantaspase finished product is a colourless to slightly yellow, clear to opalescent sterile solution for injection or infusion for intramuscular or intravenous administration. Each single-dose vial contains 10 mg of crisantaspase active substance in trehalose, sodium chloride, sodium phosphate buffer, polysorbate 80 (PS80), with a 0.5 mL nominal fill. Prior to intravenous administration, the finished product is diluted with 100 mL of normal saline solution.

Pharmaceutical development is, in general, sufficiently described. The active substance and the finished product are identical in terms of qualitative and quantitative compositions. The composition was developed to ensure suitable physicochemical and biological properties targeted to achieve the desirable safety and efficacy, considering the routes of administration, as well as product stability. A Quality Target Product Profile (QTPP) was defined by taking into consideration the pharmaceutical product quality attributes required to meet the needs of the patient.

Compatibility between the excipients and the crisantaspase active substance is considered demonstrated by the long-term stability data from both the active substance and the finished product. Selected excipients are fit for purpose and the amount of each excipient has been optimised as demonstrated by the formulation development studies. Excipients selected meet compendial requirements and are widely used in marketed products for parenteral administration. There are no novel excipients or excipients of human or animal origin.

A standard CCS configuration was chosen as a boundary condition for formulation development and was found suitable. The primary container closure system consists of a clear Type I clear borosilicate vial with a capacity of 2 mL sealed with a halobutyl rubber stopper and aluminium overseal and a violet plastic cap. Vial and stopper comply with Ph. Eur. 3.2.1 and Ph. Eur. 3.2.9, respectively. The CCS has been demonstrated to be compatible with finished product components and is suitable for intended use, based on development and process characterisation studies, as well as long-term and in-use stability data. Integrity of the finished product CCS was demonstrated.

The finished product manufacturing process has been conserved throughout development, from engineering to commercial, with only minor adjustments made to the process. The same process has been used for the manufacture of finished product batches for long-term stability and for use in clinical studies. Extensive process characterisation was performed. The data have been provided and these support the process description laid down in the dossier, including (critical) PPs with associated ranges.

The applicant provided an overview of activities to evaluate extractables and leachables potentially present in the finished product from process and product-contact equipment, components and container closure system. In general, the assessment is considered comprehensive and identified

extractables/leachables impurities are well below the acceptable limits and, therefore, do not give raise to any concerns.

The SmPC statements with respect to compatibility with syringes or with diluent and infusion sets and in-use stability for the diluted finished product for up to 12 hours at room temperature (15°C – 25°C) or 24 hours when refrigerated (2°C – 8°C) and for undiluted finished product in (polypropylene) syringes for 8 hours at room temperature or 24 hours when refrigerated (2°C – 8°C) are supported by the in-use stability study and are considered justified.

In conclusion, adequate justification is provided for the finished product formulation and manufacturing process development.

1.1.3.2. Manufacture of the product and process controls

The crisantaspase finished product is manufactured, tested and packaged in accordance with cGMP. The site responsible for batch release of the finished product is Jazz Pharmaceuticals Ireland Ltd, 5th Floor, Waterloo Exchange, Waterloo Road, Dublin 4, D04 E5W7, Ireland.

The finished product manufacturing process is a straightforward filter-fill-finish process. Both a flow diagram and a comprehensive narrative description have been provided. In summary, the process comprises of thawing and pooling of crisantaspase active substance batches, sterile filtration, filling into vials, stoppering and capering, and final 100% visual inspection. No formulation or dilution steps take place during finished product manufacture.

The manufacturing process is generally described in sufficient detail. CPPs, non-KPPs, IPCs and hold times have been defined and adequately justified. The control strategy is considered typical for this type of product and as such acceptable.

The applicant presented a traditional process validation/PPQ exercise, which elaborates on the manufacturing development data. A sufficient number of PPQ batches have been manufactured and placed on stability.

In addition to the CPPs identified, the applicant presented results/data for non-KPPs, IPCs, IPMs, finished product bulk solution homogeneity and filling consistency.

Furthermore, relevant supporting data (both product specific, e.g. hold time validation, and facility specific, e.g. media fills) have been collected. It is noted that these supporting data are often worst-case.

In conclusion, the PPQ/process verification data sufficiently support that the process can be considered validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

1.1.3.3. Product specification

The proposed release and stability specifications for the finished product are included in the dossier.

The applicant does not present an explicit discussion of the control strategy and list of tests in this section of the dossier. However, these aspects are sufficiently covered (especially with regard to product specific tests) in the active substance sections. This is considered acceptable. The majority of tests are used to control both the active substance and finished product (which is acceptable as the active substance is fully pre-formulated and little to no degradation is observed during storage), except for those which are specific for the finished product.

The specification was established based on regulatory guidelines, analytical capability, process capability and experience. For attributes tested for both active substance and finished product, results from testing of the active substance are generally used as representative of both forms as the protein concentration and matrix are identical for the two.

During the assessment, changes to acceptance criteria, in line with those requested for the active substance, were applied for the finished product specification as well. The panel of tests is considered approvable, however the applicant was asked to commit to adjust the fill weight target and change the extractable volume acceptance criterion to comply with Ph. Eur. requirements via a post-approval variation application **(Recommendation)**.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004 - Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Analytical methods

Most analytical procedures are either identical to those described for the active substance or compendial. The only non-compendial method which is unique to finished product is determination of Polysorbate 80 content, for which appropriate validation data in accordance with ICH guidelines have been provided.

Batch analysis

Batch analysis data (including PPQ batches and development batches) of the finished product were provided. The results are within the specifications applicable at the time of the release and confirm consistency of the manufacturing process.

Reference materials

Reference standards used for finished product testing are the same as those used for the active substance.

1.1.3.4. Stability of the product

The applicant claims a shelf-life for the finished product of 36 months when stored at 2-8°C.

All manufactured batches have been included in stability studies that were performed according to ICH guidelines at long-term (2-8°C), accelerated (25°C ± 2°C, 60%RH ± 5% RH) and stressed (40°C ± 2°C/75% RH ± 5% RH) conditions, using the proposed commercial primary container and closure systems. The shelf-life specifications are identical with release specification, with the exception of: identity, bacterial endotoxins and sterility (replaced by CCIT during stability), which are tested only at release.

Real-time data under long-term conditions (2-8°C) are available for 30 months for the PPQ batches and for 48 months for the development batches. Moreover, as discussed in the active substance stability section, one active substance batch displayed normal results at release, but a noteworthy degradation was observed in the stability study under accelerated and stressed conditions, which is not observed in the other batches. As the formulation and concentration of the finished product is identical to the active substance, the same atypical profile was observed in finished product stability studies under long-term, accelerated and stressed conditions. Because of this, the batch was not used for the shelf-life setting. This approach is endorsed.

To mitigate the issue, a routine stressed sample test has been implemented, with an adequate acceptance criterion, for the release of the active substance batches. As the filling operation does not offer an opportunity for the development of any new impurities, the applicant's proposal of conducting the analysis as a routine test only at active substance level is acceptable.

Photostability of the finished product was assessed on one representative batch according to the requirements of ICH Q1B. The study found only minor changes in purity due to exposure to 1.2 million lux hours of light, however no changes were observed for vials stored in cartons representative of the commercial packaging. In line with these results, the SmPC contains a warning to protect the product from light.

Overall, based on the currently available long-term stability data, no adverse trends are identified and the proposed shelf-life of 36 months for the finished product stored at 2-8°C in the commercial carton packaging can be endorsed.

As discussed in the Pharmaceutical Development section, in-use stability for intramuscular preparations in a polypropylene syringe has been demonstrated for up to 8 hours at room temperature (15°C – 25°C) or 24 hours when refrigerated (2°C – 8°C) and in-use stability for intravenous preparations has been demonstrated for up to 12 hours at room temperature (15°C – 2°C) or 24 hours when refrigerated (2°C – 8°C), from the moment of withdrawing the required volume from the unopened vials and including the 2-hour administration time.

Commitments to complete the currently ongoing stability studies and to perform post-approval annual stability studies are provided. The proposed protocol (including adequate handling of any confirmed out of specification, OOS) is acceptable.

1.1.3.5. Adventitious agents

Not applicable, as there are no sources for the presence of adventitious agents within the crisantaspase manufacturing process.

1.1.3.6. GMO

Not applicable.

1.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The applicant has applied Quality by design principles in the development of the active substance and/or finished product and their manufacturing process. However, no design spaces were claimed for the manufacturing process of the active substance, nor for the finished product.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the benefit/risk ratio of the product, which pertain to: incomplete description and/or validation of analytical procedures for determination of HCP, potency and protein concentration; need to further re-evaluate requirement for analysis of stressed active substance sample when additional batch data are available; need for adjustment of the fill weight target and change of the acceptance criteria for extractable volume in the finished product specifications to comply with Ph. Eur. requirements. These points are put forward and agreed as recommendations for future quality development.

1.1.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

1.1.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The applicant should commit to develop a new HCP test method or, alternatively, to revalidate the current HCP method as recommended in Ph. Eur. 2.6.34.
2. Concerning the method description for determination of catalytic efficiency, the applicant should commit to include additional controls to assure method validity and to comply with GMP requirements with respect to trending.
3. The applicant should commit to perform supplemental validation of the analytical method for Protein Concentration.
4. The applicant should commit to perform an evaluation of a potential potency assay method optimisation.
5. The applicant should commit to re-evaluate the requirement for conducting a stressed sample routine test for active substance release once batch data from a sufficient number of batches are available.
6. The applicant should commit to adjust the fill weight target and change the extractable volume acceptance criterion to comply with Ph. Eur. requirements via a post-approval variation application.

2.4. Non-clinical aspects

2.4.1. Introduction

JZP-458 is a new, recombinantly expressed *Erwinia chrysanthemi* asparaginase. The amino acid sequence of JZP-458 is identical to native crisantaspase. A Pseudomonas fluorescens-based platform technology was chosen as the microbial expression system for JZP-458. Following the comprehensive analytical comparability to verify that the identity, quality, and potency of JZP-458 are similar to native

crisantaspase from the natural fermentation of *Erwinia chrysanthemi* (Erwinase) in combination with the extensive clinical experience with approved asparaginases, the nonclinical strategy supporting registration of JZP-458 relies on publicly available non-clinical data on Erwinia sp. asparaginases, and is further supplemented by 4 non-clinical studies conducted with JZP-458.

2.4.2. Pharmacology

2.4.2.1. Primary pharmacodynamic studies

A cytotoxicity *in vitro* study was conducted with JZP-458 in a panel of 8 B-cell and T-cell leukaemia/lymphoma cell lines (Report 20OL766). Cells were incubated with varying concentrations of JZP-458 and luminescence measurements of ATP were used to obtain IC50 values for inhibition of cellular proliferation. All cell lines were sensitive to JZP-458 with IC50 values < 0.1 U/mL (range 0.0002-0.06 U/mL, data not shown). These results are consistent with published studies (Parmentier, 2015, Chien, 2014 Serravalle, 2016).

No additional *in vivo* pharmacology studies were submitted with JZP-458. Non-clinical *in vivo* efficacy studies using Erwinia asparaginase in mice or rats are reported in the published literature and studies demonstrate the *in vivo* pharmacological activity of *Erwinia* sp. asparaginase in mouse or rat tumour models, at doses that were associated with depletion of plasma asparagine.

2.4.2.2. Secondary pharmacodynamic studies

An overview of literature was provided by the applicant, which demonstrates activity of asparaginases in depleting asparagine content in blood plasma, reducing tumour burden and increasing survival times in mouse or rat tumour models (Minetto, 2017; Chen 2017; Sanghez, 2018; Chiu, 2014). These effects are most likely mediated through the depletion of asparagine, which is the primary mode of action. Immunosuppressive effects of asparaginase were also described by reducing macrophage activity in clinical use (Song, 2017). Further evidence of immunosuppression was provided as *Erwinia* carotovora was able to inhibit the blastogenic response of both rabbit and human leukocytes *in vitro* (Ashworth, 1974; Han, 1972)., and reduced antigenic response after a challenge with sheep red blood cells in mice (Berenbaum, 1970).

2.4.2.3. Safety pharmacology programme

No safety pharmacology data was submitted for JZD-458 or asparaginase. Since asparaginase is derived from biotechnological processes, stand-alone safety pharmacology studies are not needed, in line with ICH S6(R1).

2.4.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies on *Erwinia* asparaginases were identified in the published preclinical literature.

2.4.3. Pharmacokinetics

Absorption

The pharmacokinetics of JZP-458 were evaluated in a single-dose mouse and a single dose rat study after IV administration (Report WIL-350510), in comparison with several other (mostly conjugated) asparaginases, including two control asparaginases in rat (Oncaspar and pegcrisantaspase).

In mice and rat, although dose normalised Cmax was comparable to the other asparaginases, DN AUC was 8-15 times lower (except for recombinant crisantaspase from Corynebacterium). In addition, clearance was 20.4 ml/hr/kg in mice and 9.82 ml/hr/kg in rat, which is 8-14 (mice) or 11-18 (rat) times higher than for the other asparaginases. T1/2 (2.13h in mice and 1.35h in rat), although similar to literature data from *Erwinia* asparaginases, was 8-13 (mice) and 23-41 (rat) times lower than for the other asparaginases, including the control substances. It is noted that, besides T1/2, no other comparisons were provided for the PK parameters of other asparaginases in literature. Based on the provided data, to result in similar AUC values, JZP-458 should therefore be administered at much higher doses than the other asparaginases, resulting in higher Cmax values (or more frequent administration of lower doses to prevent a higher Cmax). Nevertheless, no adverse effects due to a higher Cmax were observed in the clinical studies.

Volume of distribution was comparable to the other asparaginases tested in mice (56.9 ml/kg) and slightly (2-3times) lower in rats (16.4 ml/kg) and indicates a distribution confined to the central vascular compartment.

After repeated dosing for 14 days in rats the increase in Cmax was dose-proportional. The increase in AUC was slightly more than dose proportional. No accumulation and no gender differences were observed. Dose levels used in the repeated dose study were 12-120 times higher than in the single dose PK study. Clearance (19.1 to 35.1 mL/h/kg) and VSS (69.1 to 100 mL/kg) were 2-6 times higher than after the lower dose used in the single dose study.

Distribution

Formal tissue distribution and protein binding studies were not conducted with JZP-458 in accordance with regulatory guidelines for biotechnology-derived pharmaceuticals. JZP-458 has a low volume of distribution (16.4-56.9/kg in rat and mice, respectively), suggesting a distribution limited to the central vascular compartment.

Neither placental transfer nor excretion to milk have been investigated.

Metabolism

No metabolism studies with JZP-458 were conducted in animals. The absence of metabolism studies is in accordance with ICH S6(R1) and is agreed with. Recombinant crisantaspase is expected to be metabolised into small peptides by catabolic pathways.

Excretion

No excretion studies with JZP-458 were conducted in animals. The absence of excretion studies is in accordance with ICH S6(R1).

Immunogenicity

In the repeat dose toxicity study with JZP-458 (Study 2018-458-032), the incidence of ADA induction was 0% (0 of 30) in the control group, 45% (9/20) at 3000 U/kg/day, 5% (1 of 20) at 10,000 U/kg/day, and 20% (6 of 30, all from the recovery animals) at 30,000 U/kg/day. This is comparable

with the results from studies conducted with *Erwinia chrysanthemi*-derived asparaginase and can be expected considering the bacterial origin of the asparaginase.

2.4.4. Toxicology

2.4.4.1. Single dose toxicity

Based on studies from the early 1970s, asparaginase administered as a single IV dose up to 5000 IU/kg appears to be relatively well tolerated in rats, hamsters and rabbits (SSARL-DPH-72-05). In contrast, in another study (SSARL-DPH-72-02), doses of 2000 IU/kg and onwards were lethal to rabbits. This difference was not explored further by the applicant. However, these studies are decades old and were not conducted under GLP conditions and are thus only considered to be supportive for this application.

2.4.4.2. Repeat dose toxicity

A 14-day repeat dose toxicity study in rat was performed with JZP-458 (Report 2018-458-032). Rats received daily infusion of JZP-458 at doses of 0, 3,000, 10,000, and 30,000 U/kg/day JZP-458 (corresponding to 0, 4.6, 15.2, and 45.6 mg/kg respectively). JZD-458 was immunogenic with approximately half of all animals in the low-dose recovery group having a positive anti-drug antibody (ADA) titer. For the high and mid dose group the incidence was limited. Immunogenicity did not affect the interpretation of the study.

The high-dose of 30,000 U/kg/day was poorly tolerated with animals being euthanised or put in early recovery on day 6. Animals in this group suffered severe weight loss, piloerection, rough coat and oral discharge. Weight loss, markedly lower reticulocytes and moderately decreased platelet counts were noted in addition to haematological changes observed in lower dose groups. Mild elevation of blood glucose and blood urea nitrogen (BUN) was observed in this group as well as and minimal to mild increases in total protein, albumin and globulin. High dose group animals had slight to marked decrease in splenic red pulp and extramedullary haematopoiesis and slight to severe decreased cortical lymphocytes of thymus.

In surviving animals of the high dose group, all findings were reversible.

Dose-dependent decrease in food consumption and body weight was observed in animals given 3,000 U/kg/day onwards. In the 10,000 U/kg/day group this was also accompanied by piloerection. Histopathological changes from 3,000 UI/kg/day onward included decreased erythroid parameters, decreased lymphocytes and increased myeloid precursors in bone marrow. Spleen weights were decreased, corresponding with which was accompanied by minimally or slightly lower extramedullary haematopoiesis.

The NOAEL was 10,000 U/kg/day, which corresponds to an exposure of 322,000 h.mIU/ml. This is approximately 3.6 times the human MRHD (based on 48 hour IV dosing AUC of 179 h.U/ml). The low exposure multiple suggests that the observed toxicity can be clinically relevant. Given the extensive clinical experience with asparaginases, this assessment is considered to be adequate.

Historical repeat dose toxicity studies in rabbits and monkeys, which were not conducted under GLP conditions, showed that in rabbits, 1,000 IU/kg/day was associated with changes in clinical chemistry (BUN, glucose, creatinine, liver enzymes), haematology, and histopathology (liver, and kidney). In monkeys, doses up to 10,000 IU/kg *Erwinia* sp. asparaginases were generally tolerated. No

toxicologically meaningful changes were observed at 1,000 IU/kg. At 2,000 and 10,000 IU/kg/day, hepatocellular vacuolation, transient leukopaenia, and increases in serum triglycerides, lipids, and glucose were observed.

2.4.4.3. Genotoxicity

In line with ICH S6(R1) and ICH S9, no genotoxicity studies were submitted.

2.4.4.4. Carcinogenicity

In line with ICH S6(R1) and ICH S9, no carcinogenicity studies were submitted.

2.4.4.5. Reproductive and developmental toxicity

No reproductive or developmental toxicity studies were performed with JZP-458. GLP-compliant reproductive and developmental toxicity studies submitted were conducted with Erwinaze (asparaginase *Erwinia chrysanthemi*)

In a GLP fertility and early embryonic development study (Study 11-4368) in which male rats were dosed from 4 weeks before mating until 25 days after the end of the mating period and females were dosed from 2 weeks before mating until GD 6, a decrease in sperm count was noted in males, but there were no effects on fertility parameters at any doses. Therefore, the NOAEL for systemic and fertility effects in both male and female rats was 2,000 IU/kg IM QOD (every other day). At the end of the study, all Erwinaze/Erwinase-treated animals were positive for ADAs.

In a GLP embryofoetal toxicity study in which mated female rats were dosed during the period of organogenesis (GD 6-GD 16), the maternal systemic NOAEL was 1,000 IU/kg IM QOD due to decreased body weights and food consumption. Based on the slight increase in partially undescended thymic tissue in the 2,000 IU/kg dose group, the foetal NOAEL was also considered to be 1,000 IU/kg IM QOD. There were no effects on the reproductive parameters and thus the reproductive NOAEL was 2,000 IU/kg IM QOD. In this study, 26 of the 27 (maternal) rats assessed for ADAs on GD 20 were positive, of which 18 were positive for neutralizing ADAs.

In a GLP embryofoetal toxicity study in which mated female rabbits were dosed during the period of organogenesis (GD 6–GD 18), the maternal systemic NOAEL was 10 IU/kg IM QOD due to decreased body weight and food consumption. Neither the maternal reproductive nor the foetal NOAELs could be determined due to increased resorptions and post-implantation loss, fewer live foetuses, decreased gravid uterine and foetal/litter weights, foetal visceral abnormalities, and delays in foetal skeletal ossification. At the end of the study (GD 29), 8 of the 9 rabbits evaluated for ADAs were positive, of which 5 were positive for neutralizing ADAs.

In a pre- and postnatal developmental toxicity study in which pregnant and lactating female rats were dosed from GD 6 through weening of their offspring (of which selected pups were evaluated through sexual maturity, mating, and establishment of pregnancy), there were no effects on gestation, delivery, viability and lactation indices, pup body weight or clinical signs during and after the lactation period. There was a slight Erwinase-related increase in time to acquire surface righting ($\geq$ 600 IU/kg) and mid-air righting ($\geq$ 1,200 IU/kg), but the findings were considered non-adverse. Therefore, the maternal and offspring (pre- and postnatal development) NOAEL was 2,400 IU/kg IM QOD. ADAs were not evaluated in this study.

Although there were no effects on the reproductive parameters or significant foetal observations in rats in reproductive and developmental studies with Erwinaze/Erwinase, the presence of neutralizing ADAs

complicates the interpretation of these studies. In addition, there were findings in the rabbit developmental toxicity study, despite the presence of ADAs. Based on these observations JZP-458 should be considered a potential reproductive and developmental toxicant. This has been adequately reflected in the SPC.

Based on the extensive clinical experience available for crisantaspase and its well documented safety profile, juvenile toxicology studies with JZP-458 were not warranted.

2.4.4.6. Toxicokinetic data

Plasma concentrations increased approximately dose proportionally (Cmax) to slightly (1.2-1.8x) more than dose proportional (AUC) in the repeat dose toxicity study with JZP-458.

Exposure multiples based on Cmax and AUC0-24 were 3.7 and 3.6 (based on the maximum Cmax [21.6 U/ml] and AUC0-48 [182 U·h/ml] observed for the clinical 25/25/50 mg/m2 schedule on Monday/Wednesday/Friday or the 25 mg/m2 schedule every 48 hours).

The pharmacokinetics of JZP-458 were studied primarily in mice and rats and only following IV administration. Based on the low Vss (56.9 ml/kg in mice, 16.4 ml/kg in rats and 1.40 L/m2 in humans), distribution was limited to the central vascular compartment. Plasma clearance in animals is low (20.4 ml/hr/kg in mice and 9.82 ml/hr/kg in rat), as well as in humans (0.12 L/hour/m2). Following repeated dosing (14 days in rats) an approximately dose proportional increase in exposure was observed for Cmax and AUC (dose levels 3000-30000 U/kg) as well as in humans at doses from 12.5 to 50 mg/m2. No plasma AUC accumulation following repeated dosing of JZP-458 was observed. No gender differences were observed in the pharmacokinetics of JZP-458. The elimination half-life following IV administration was 2.13h in mice,1.35h in rat, and 8.27 hours in humans. After IM administration, elimination half-life in humans was twice as long (19.1h).

2.4.4.7. Local tolerance

An assessment of local tolerance was incorporated in the repeat dose toxicity study with JZP-458 in rats. No adverse findings local tolerance findings were observed. Experience with other asparaginases does not suggest local tolerance to be a risk factor.

2.4.4.8. Other toxicity studies

In an investigative study of the diabetogenic potential of *Erwinia* sp. Asparaginase (SSARL-DPH-70-07), there was no definitive evidence of diabetogenic potential or fatty liver and bromosulfophthalein retention following daily administration of 2000 IU/kg Erwinia sp. asparaginase for 5 days to 1 monkey or of a single dose of 10,000 IU/kg to 1 rabbit.

2.4.5. Ecotoxicity/environmental risk assessment

In line with the guideline on the Environmental Risk Assessment for medicinal products for human use and Questions and Answers on the guideline (EMEA/CHMP/SWP/4447/00 corr 2), and because JZP-458 is derived from biotechnological processes, a formal ERA is not required.

2.4.6. Discussion on non-clinical aspects

Considering the comparability of JZP-458 with Erwinase, a standard nonclinical package was not provided. Instead an integrated non-clinical strategy was used, consisting of publicly available non-clinical information on *Erwinia* sp. asparaginases, further supplemented by 4 non-clinical studies conducted with JZP-458. This was considered acceptable.

Submitted *in vitro* studies demonstrate potent anti-proliferative effects of JZP-458 in leukaemia/lymphoma cell lines with IC50 values that are lower than the nadir SAA levels of ≥0.1 IU/mL achieved during clinical use of JZP-458. The *in vitro* data obtained with JZP-458 in leukaemia/lymphoma cell lines are generally consistent with the published literature for other *Erwinia*-derived asparaginases. Inhibition of proliferation was consistent with that reported in literature suggesting that JZP-458 is comparable to other asparaginases, thus providing adequate demonstration of the proof of concept *in vitro.*

Information on the secondary pharmacology of asparaginase is provided through literature data. Due to the comparable activity of JZP-458 and other asparaginases, animal studies would have likely resulted in comparable results as well and these studies would have added no real evidence to support the MAA of JZP-458. Literature data is also not suggestive of any safety pharmacology related issues.

From the pharmacokinetic point of view, the rat was the most relevant species for non-clinical efficacy and safety studies. Overall, pharmacokinetics has been sufficiently described following the IV route.

The toxicology programme revealed adverse effects fully comparable to other asparaginases at clinically relevant doses, which were primarily restricted to haematological effects. At higher doses, the clinical condition of test animals rapidly deteriorates. The low exposure multiples (3.6 times above the maximum human exposure) suggest that the observed toxicity, which is typical of asparaginases, may be clinically relevant and should be monitored clinically (see clinical aspects and discussion).

In a fertility and early embryonic development study in rats with *Erwinia chrysanthemi* crisantaspase, there were no effect on female or male fertility. In embryofoetal development studies in rats and rabbits, *Erwinia chrysanthemi* L-asparaginase produced maternal toxicity, increased resorptions, post implantation loss, embryofoetal toxicity, and/or gross abnormalities at exposures lower than those observed clinically.

2.4.7. Conclusion on the non-clinical aspects

From a non-clinical point of view Enrylaze the efficacy and safety profile of has been adequately characterised and is recommended for marketing authorisation.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

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

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Study Number/ Country	Study Design/ Status	Product, Route, Dose, and Duration of Treatment/ Lot Number	Participant Population; Age
JZP458-101	Phase 1, randomized, single-center, open-label study to evaluate the safety, tolerability, and PK of a single dose of JZP-458 via either a 2-hour infusion or IM administration in healthy adult participants. Included Erwinaze IV and IM cohorts. Completed	Single dose JZP-458: IV doses of 25 and 37.5 mg/m^2, and IM doses of 25 and 12.5 mg/m^2 Lot number for IV and IM JZP-458: AK0926B Single dose Erwinaze: IV and IM doses of 25,000 IU/m^2 Lot number for Erwinaze: 189K118	Enrolled: n=30; Age range: 25 to 52 years Treated: n=30 (19 male, 11 female)
JZP458-201 (Interim analysis of data as of 19 July 2021)	Phase 2/3, open-label, multicenter, dose confirmation, efficacy, safety, and PK study of JZP-458 in participants (of any age) with ALL/LBL who are hypersensitive to *E. coli*-derived asparaginases (allergic reaction or silent inactivation). Part A (IM): Ongoing, enrollment closed Part B (IV): Ongoing, enrollment closed	Part A: Cohort 1a: Multiple doses of IM JZP-458 25 mg/m^2 administered MWF Cohort 1b: Multiple doses of IM JZP-458 37.5 mg/m^2 administered MWF Cohort 1c: Multiple doses of IM JZP-458 25/25/50 mg/m^2 administered MWF[a] Part B: Cohort 1a: Multiple doses of IV JZP-458 25/25/50 mg/m^2 administered MWF[a] Lot numbers for IM JZP-458 as of 19 July 2021: AK0926B, AL4753B, AM0383B, and AN8014B	Included in analysis: Part A: Cohort 1a: Enrolled and treated: N = 33 (16 females; 17 males); Age range: 1 to 24 years Cohort 1b: Enrolled and treated: N = 83 (28 females; 55 males); Age range: 1 to 20 years Cohort 1c: Enrolled and treated: N = 51 (20 females; 31 males); Age range: 3 to 25 years Part B: Cohort 1a: Enrolled and treated: N = 61 (25 females; 36 males); Age range: 1 to 24 years

Abbreviations: ALL = acute lymphoblastic leukemia; IM = intramuscular; IV = intravenous; LBL = lymphoblastic leukemia; MWF = Monday, Wednesday, Friday; PK = pharmacokinetics

[a] 25 mg/m^2 JZP-458 administered IM on Monday and Wednesday, and 50 mg/m^2 JZP-458 administered IM on Friday

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

The clinical pharmacology development programme for RC-P includes 2 studies: a completed phase 1 study in healthy adult participants (JZP458-101) and an ongoing pivotal phase 2/3 study (JZP458-201) in paediatric and adult participants with ALL/LBL who have developed hypersensitivity to *E. coli*-derived asparaginases. A popPK analysis of data from Study JZP458-101 in healthy participants was performed to facilitate the selection of a starting dose for the phase 2/3 pivotal Study JZP458-201.

Population PK (PopPK)analyses of data from Study JZP458-201 in participants with ALL/LBL were performed to support dose selection and dose confirmation throughout the study.

PopPK models

Two popPK models were developed during phase 2/3 of the development programme, i.e., an IM and IVIM popPK model, in support of the various treatment regimens. The IM popPK model uses only a subset of the data for model development (only sparse IM SAA PK data from Study JZP458-201 were included). The IVIM popPK model contains all (available at the time) data from phase I and phase 2/3 studies.

The IVIM popPK model was a 1-compartment model with sequential mixed-order absorption for IM (parameters estimated include: F1, zero order R1 followed by a 1st order absorption KA), linear elimination with population estimate for CL fixed to the value obtained from the IV alone model; inter-individual variability (IIV) expressed as an exponential term on CL, V, R1, and KA; off-diagonal covariance term for CL and V; and APEM residual error models with M3 BLQ data handling method that were described separately for IM and IV data. BSA was included as a significant covariate on CL and V. The covariate analysis also identified race (African American) on CL, disease (ALL/LBL) on CL for patients following IM administration, and disease (ALL/LBL) on V as significant covariates. African Americans had 25% lower CL, ALL/LBL patients who received IM administration had 104% higher (2.04 fold) CL, and ALL/LBL patients had 24% lower V than healthy volunteers. There were no differences in CL between Hispanic and Non-Hispanic study participants. The BSA standard used for scaling was 1.2 m^2 to be reflective of an average paediatric study participant.

During evaluation of this application, the model was updated to incorporate the final data from study JZP458-201 (final database lock date of 22 Nov 2022). During the initial modelling, the eta distributions suggested that the effect of healthy volunteers versus patients should be included in the base model. In addition, body size, as measured by body surface area (BSA), was also incorporated to stabilise the model further. The condition number was checked to ensure that no correlation was introduced at this point. In addition, an effect of patient age was also included on the first absorption rate constant (KA1) in the CP model.

Further examination showed that the between-subject variability (BSV) on V had a high degree of shrinkage, and several steps were taken to resolve this problem. This included testing a Manly transform for BSV on V and several OMEGA BLOCK models. The transform did not appreciably reduce the shrinkage but did show that the BSV on CL and V were highly correlated (0.8), and a shared eta approach between CL and V was used instead. This approach necessitated fixing BSV on V to 0 and removing V from MU modelling, this then resulted in a model that converged with acceptable parameter precision and removed the problem of high correlation. In addition, the BSV on the second absorption rate constant (KA2) was poorly estimated and fixed to a small value (10%) to improve stability further.

This model then became the 'base model', and other covariates were examined graphically using eta plots and were subsequently tested as being potentially influential as single covariate models, including anti-drug antibody (ADA), neutralizing antibody (NAB), RACE (each race was tested singly), disease subtype and primary disease (B CELL ALL, T CELL ALL, B CELL LBL, T CELL LBL, B CELL, T CELL, ALL and LBL were all tested singly). In addition, the dose (ADOSE) was tested on CL, V, and F1 but found to be important only on F1. Other covariates were identified but came out of the model on back elimination or were small enough to have no clinical relevance (a change in parameter over 20% is the usual cut-off for relevance). The only covariate further identified was the effect of RACE 2 (BLACK/AFRICAN AMERICAN) on CL. No other covariate evaluated provided a substantial decrease ($P < 0.05$) in the IMP objective function (OBJ) or was estimated with a clinically meaningful change in the parameters with sufficient precision to be included in the model. The final model evaluation determined that the dose effect on F1 had a deleterious effect on the precision for CL, so this factor was also removed.

The final IVIM popPK model was used to simulate SAA profiles to predict the proportion of subjects achieving a therapeutic nadir SAA (NSAA) level ≥0.1 IU/mL at 48 and 72-hours post-dose using different IV and IVIM-combined dosing regimens (**Table 28**). Table 26 shows each regimen's Cmax and Ctrough values.

Table 2. Overall comparison of observed and simulated response rate of achieving a therapeutic NSAA level ⩾ 0.1 IU/mL following different dosing schedules (IVIM popPK model)

Response Rate (NSAA ≥ 0.1 IU/mL)	Time from Last Dose	Observed % [95% CI]	Simulated NHANES % [95% CI]
25 mg/m^2 IM MWF × 6	48 hours	96.9% [90.8%, 100%]	95.0% [94.0%, 95.9%]
	72 hours	64.3% [46.5%, 82.0%]	69.2% [67.1%, 71.2%]
37.5 mg/m^2 IM MWF × 6	48 hours	98.8% [96.4%, 100%]	98.8% [98.3%, 99.2%]
	72 hours	90.9% [84.5%, 97.3%]	82.8% [81.1%, 84.5%]
25/25/50 mg/m^2 IM MWF × 6	48 hours after 25 mg/m^2	95.9% [90.4, 100%]	94.6% [93.6%, 95.6%]
	72 hours after 50 mg/m^2	89.8% [81.3%, 98.3%]	88.5% [87.1%, 89.9%]
25 mg/m^2 IM Q48h × 7	48 hours	NA	96.7% [95.9%, 97.4%]
25/25/50 mg/m^2 IV MWF × 6	48 hours after 25 mg/m^2	89.8% [82.1%, 97.5%[	84.0% [82.4%, 85.6%]
	72 hours after 50 mg/m^2	40.0% [26.4%, 53.6%]	38.2% [36.1%, 40.4%]
25 mg/m^2 IV Q48h × 7	48 hours	NA	83.4% [81.7%, 85.0%]
25/25/50 mg/m^2 IV/IV/IM MWF × 6	48 hours after 25 mg/m^2	NA	89.5% [88.1%, 90.8%]
	72 hours after 50 mg/m^2	NA	88.8% [87.4%, 90.1%]

Abbreviations: CI = confidence interval; IM = intramuscular; IV = intravenous; IU = international units; MWF = Monday, Wednesday, and Friday; NA = not applicable; NHANES = National Health and Nutrition Examination Survey; NSAA = nadir serum asparaginase activity.

Table 3. RC-P pharmacokinetic parameters based on SAA

PK Parameter	Geometric mean (% Geometric CV) after last dose							
	25 mg/m² Every 48 hours	25 mg/m² Every 48 hours	25/25/50 mg/m² Monday, Wednesday, Friday		25/25/50 mg/m² Monday, Wednesday, Friday		25/25/50 mg/m² Monday, Wednesday, Friday	
			Last 25 mg/m²	Last 50 mg/m²	Last 25 mg/m²	Last 50 mg/m²	Last 25 mg/m² IV	Last 50 mg/m² IM
	Intramuscularly	Intravenously	Intramuscularly		Intravenously		IV/IV/IM	
C_{max} (U/mL)	0.79 (87%)	8.44 (62%)	0.78 (88%)	1.09 (98%)	8.62 (62%)	16.98 (62%)	8.71 (61%)	0.90 (73%)
C_{trough} (U/mL)	0.48 (98%)	0.23 (107%)	0.42 (99%)	0.27 (105%)	0.24 (106%)	0.07 (155%)	0.28 (106%)	0.24 (99%)

Absorption

Absorption of RC-P was studied in Study JZP458-101 and was evaluated using popPK modelling. RC-P is slowly absorbed following IM administration. The median of individual popPK predicted Tmax of RC-P is 14.95 hours, whereas Tmax reported in Study JZP458-101 is 24.00 hours (range: 24.00 - 36.00 hours). The mean absolute bioavailability for intramuscular administration is 34.5-36.8% in healthy subjects.

Distribution

Distribution of RC-P was evaluated using popPK modelling and in Study JZP458-101. Following IM administration (IM popPK model), the geometric mean (%CV) of individual predicted V/F of RC-P is 1.14 L/m^2 (72%). Following IV administration (IVIM popPK model), the geometric mean (%CV) of individual predicted V of RC-P is 1.40 L/m^2 (23%). In the updated popPK model V was estimated to be 1.7 L/m^2. Following intravenous administration, the mean (%CV) volume of distribution of recombinant crisantaspase is 1.94 L (22.4%) and 1.79 L (16.6%) following dose of 25 mg/m$_2$ and 37.5 mg/m$_2$, respectively, in healthy subjects.

Elimination

Elimination of RC-P was evaluated using popPK modelling and in Study JZP458-101. Following IM administration (IM popPK model), the geometric mean (%CV) of individual predicted CL/F of RC-P is 0.31 L/h/m^2 (45%) and the t½ is 19.1 hours (3%). Following IV administration (IVIM popPK model), the geometric mean (%CV) CL of RC-P is 0.117 L/hour/m^2 (20%) and the t½ is 8.27 hours (15%). RC-P is an enzyme and it is therefore expected to be metabolised by proteolytic degradation. In the updated popPK model CL was estimated to be 0.14 L/h/m^2 (20%).

Following intravenous administration, the mean (%CV) clearance of recombinant crisantaspase is 0.125 L/h (22.4%) and 0.107 L/h (16.6%) following dose of 25 mg/m$_2$ and 37.5 mg/m$_2$, respectively, in healthy subjects.

The mean (%CV) half-life is 11.5-12.6 hours (11.2-12.8%) following intravenous administration and 19.1-23.4 hours (21.8-23.6%) following intramuscular administration in healthy subjects.

Dose proportionality and time dependencies

Dose proportionality

In Study JZP458-101, RC-P exposures (SAA Cmax and AUC) increased to an approximately proportional extend with increasing doses within the dose range of 12.5 to 25 mg/m^2 for IM administration, and within the dose range of 25 to 37.5 mg/m^2 for IV administration. In Study JZP458-201, based on initial popPK analyses, model-predicted exposure parameters (SAA Cmax and AUC) were approximately dose proportional between the dose range of 25 to 50 mg/m^2 for IM and IV administrations.

Time dependency

In Study JZP458-201, based on a dosing frequency of once every 48 hours and t½ of 19.1 hours for IM and 8.27 hours for IV, the accumulation ratio for IM and IV RC-P is 1.21 and 1.02, respectively. The initial popPK model-predicted exposures parameters (SAA Cmax and AUC) generated for RC-P following multiple dosing also suggest that there was little accumulation.

Special populations

No dedicated PK studies were performed in special patient populations except paediatrics, though PK analysis was used to investigate SAA-time profiles/PK in sub-groups of patients.

Renal and hepatic impairment

RC-P is an enzyme with a molecular weight of 140 kDa, which due to its size is most likely not renally eliminated. Therefore, the effect of renal impairment is unlikely to result in changes in the SAA-time profiles/PK. Likewise, the enzyme is not metabolised by hepatic enzymes and hepatic impairment is unlikely to alter RC-P SAA-time profiles/PK.

Race, age and body surface area (BSA)

PopPK simulated RC-P SAA-time profiles were compared across different subcategories within race (White, African American, Asian, and other), age (1 month to < 2, 2 to < 6, 6 to < 12, 12 to < 18, 18 to < 65, and 65 to 80 years), and BSA (0.25 to < 1.00, 1.00 to < 1.66, 1.66 to < 1.95, 1.95 to 3.03 m^2). In all situations, the simulated proportion of participants achieving NSAA $\geq$ 0.1 IU/mL was around 90%, which, according to the applicant, would suggest that no clinically significant difference is expected in the probability of achieving a therapeutic NSAA based on race, age, or BSA, following the proposed BSA-based dosing regimens. African Americans had 25% lower clearance, however subgroup analysis on race with NHANES suggested that the SAA time profiles were also comparable and largely overlapping across different race groups. This conclusion could also be supported based on observed PK parameters independent of the popPK model.

Weight and gender

Subgroup analysis for the assessment of the impact of gender and weight on exposure has not been performed. This was performed with the updated popPK model and no significant effect was identified. This conclusion could also be supported based on observed PK parameters independent of the popPK model.

Elderly and young children

No elderly subjects (maximum age 52 years) or very young subjects (younger than 1 year) were included in the clinical studies. The clinical data obtained in the phase 2/3 study is obtained from children (age range 1 – 25 years of age).

Pharmacokinetic interaction studies

The applicant indicates that DDIs are unlikely as RC-P is an enzyme and is not metabolised by hepatic cytochrome P450 enzymes. No dedicated DDI study has therefore been conducted.

2.5.2.2. Pharmacodynamics

Mechanism of action

Asparaginase *Erwinia chrysanthemi* (recombinant) catalyses the conversion of the amino acid L-asparagine into aspartic acid and ammonia. The mechanism of action of JZP-458 is thought to be based on the inability of leukemic cells to synthesise asparagine due to lack of asparagine synthetase activity, resulting in cytotoxicity specific for leukemic cells that rely on exogenous asparagine for their protein metabolism and survival.

JZP-458 has the identical amino acid sequence as native crisantaspase.

Primary and Secondary pharmacology

Pharmacodynamic endpoints (L-asparagine and L-glutamine concentrations) have been evaluated in pivotal clinical Phase 2/3 Study JZP458-201. For a description of the design of Study JZP458-201, please refer to the clinical efficacy section of this report.

For 49% of patients, complete asparagine depletion, (asparagine levels < 0.025 µg/mL, below the lower limit of quantification (LLOQ) for all 7 postdose samples collected in Course 1), was obtained following IM administration of doses at 25 mg/m^2 MWF, 37.5 mg/m^2 MWF, and IM or IV 25/25/50 mg/m^2 MWF. Nearly complete depletion (asparagine levels < 0.05 µg/mL, or 2 times the LLOQ, for all 7 postdose samples collected in Course 1) was obtained in ~80% of patients. Glutamine levels were only moderately affected.

Relationship between plasma concentration and effect

The objective of the PK/PD models was to identify correlations between SAA and: 1) selected pharmacodynamic markers (PD) and 2) safety signals.

Logistic regression (PK-PD)

The relationship between SAA parameter AUC336 and the incidence of L-asparagine or L-glutamine depletion at the last sample (48h or 72h, as available per study participant and regimen) for Course 1 was assessed using logistic regression. This analysis indicated that AUC336 was not strongly related to L-asparagine/L-glutamine depletion. The same logistic regression analysis was carried to assess the relationship between time-matched SAA values and the incidence of L-asparagine or L-glutamine depletion. A significant relationship between SAA and L-asparagine/L-glutamine depletion was observed.

Logistic regression (PK-safety)

The relationship between model predicted SAA parameters from Study JZP458-201 Course 1 (Cmax,6, AUC336, C48,L, C72,L) and the incidence of selected drug-related adverse events (hypersensitivity, hepatotoxicity, pancreatitis, thrombosis, and hypertriglyceridemia) from Course 1 and during study (all Courses) was evaluated using logistic regression. There was a statistically significant relationships between the exposure PK parameters and hypersensitivity.

2.5.3. Discussion on clinical pharmacology

Pharmacokinetics

Two clinical studies have been initiated where RC-P PK samples have been taken, which contribute to the clinical pharmacology programme. In the completed Phase 1 study, PK has been studied after single dose IV (25 and 37.5 mg/m^2) and IM (12.5 and 25 mg/m^2). A dense sample scheme has been used up until 96 hours after dosing. In addition, sparse PK samples are gathered in an ongoing, open label Phase 2/3 study at 2 hours (IV)/2.5 hours (IM), 48 hours or 72 hours after dosing dependent on the dose number and the specific treatment course. The Phase 1 and Phase 2/3 studies appear sufficient to characterise the PK profile of RC-P.

PopPK

The applicant used popPK to extrapolate to different dosage regimens that were not specifically tested in the phase 2/3 study. PopPK in principle can be used for this purpose, in case a suitable model is developed, and a clear target is present. The target indicated by the applicant: NSAA level ≥ 0.1 IU/mL at the last 72-hour time point in the first course of treatment, is adequate. It is not clear however if the model submitted by the applicant is suitable for its intended purpose. A flexible change point[(CP) MTIME] model was implemented on absorption parameters. Parameterisation of absorption with change point is considered to have no physiological basis and is therefore questionable. In addition, Predictive-corrected visual predictive check (pcVPCs) which were submitted are difficult to interpret due to the time scale used. Consequently, the popPK model is not considered as pivotal evidence in this application, and conclusions will be based on pharmacokinetic studies.

The model cannot be used to predict target achievement for the dosing regimen not tested in the clinical studies. However, the dosing regimens that were not tested are very similar to those tested in the clinical studies. Of note, all doses in the new regimens (25 mg/m2 IM, 25 mg/m2 IV, and 50 mg/m2 IM) and dose-intervals in the new regimens (48h or 72 h) are also present in one of the dose regimens that were tested in the clinical studies. This is therefore considered an interpolation. Although no exact numbers on response rate can be calculated, from pharmacokinetic principles no (large) differences in exposure and response rate are expected for regimens not tested in clinical studies. Importantly, SAA levels can be monitored in clinical practice.

ADME

The median Tmax of recombinant crisantaspase is 14.95 hours following intramuscular administration. The mean absolute bioavailability for intramuscular administration is 37% in healthy subjects.

Following intravenous administration, the geometric mean (%CV) volume of distribution of recombinant crisantaspase is 1.75 L/m2 (14%).

Recombinant crisantaspase is expected to be metabolised into small peptides by catabolic pathways.

Following intravenous administration, the geometric mean (%CV) clearance of recombinant crisantaspase is 0.14 L/hour/m2 (20%).

The geometric mean (%CV) half-life is 8.6 hours (13%) following intravenous administration and 18.8 hours (11%) following intramuscular administration.

There was no dedicated study on renal or hepatic impairment with Enrylaze. However, renal and hepatic impairment were evaluated within the PPK analysis and PK analysis in subgroups.

During treatment, dose adjustment is not required for patients with total bilirubin ≤3 times the Upper Limit of Normal; there is limited data with Enrylaze in patients with total bilirubin > 3 times to ≤ 10 times the Upper Limit of Normal (ULN).

Dose adjustment is not required for patients with pre-existing mild or moderate hepatic impairment (total bilirubin >1 to 3 times the ULN or AST > than the ULN). There are insufficient data in patients with pre-existing severe hepatic impairment to support a dose recommendation. There are insufficient data in patients with mild, moderate or severe renal impairment to support a dose recommendation.

There were no clinically significant differences in the pharmacokinetics of RC-P based on weight (9 to 131 kg) or sex (n=138 male; n=88 female) after the dose was adjusted by body surface area (BSA).

The volume of distribution and clearance of recombinant crisantaspase increase with increasing BSA (0.44 to 2.53 m2).

Age impacts absorption rate constant whereas younger subjects have higher absorption rate constant value, leading to earlier Tmax. The phase 2/3 Study JZP458-201 submitted by the applicant included children between 1 and 25 years of age. As elimination is not mediated by specific CYP enzymes or renal function large effects of developmental processes in these pathways on exposure are not expected for the older age groups. In addition, for other asparaginase products no dose adjustments based on older age are required. It is important to note that SAA levels can be monitored in clinical practice.

Black or African American patients had 25% lower clearance which may increase SAA exposure compared to population average. No dose adjustment is needed in African American population. There were no clinically significant differences in clearance between Hispanic and Non-Hispanic patients.

No drug-drug interaction study has been performed. DDIs are unlikely due to the elimination route being proteolytic catabolism. In the SmPC it is stated that RC-P might increase toxicity of other medicinal products through its effect on liver function. Additional information on potential drug interactions with medications commonly used in combination with asparaginase in the treatment of ALL/LBL (vincristine, methotrexate, cytarabine and other glucocorticoids) from other asparaginase containing products has also been included in the SmPC for Enrylaze.

Pharmacodynamics

While 49% of all IM + IV patients obtained complete asparagine completion, ~80% obtained nearly complete depletion. Data seem to support efficacy of RC-P treatment, but interpretation is hampered due to *ex vivo* hydrolysis by asparaginase.

The absence of complete glutamine depletion with only moderate affection of glutamine levels and complete depletion of asparagine suggest an acceptable low ratio of L-glutaminase/L-asparaginase activity.

A significant relationship between SAA and L-asparagine/L-glutamine depletion was observed using logistic regression.

Based on observed IM data, 4 ADA positive participants with lower SAA levels (< 0.1 IU/mL) at matching time points in Course 1 were observed. The SAA and SAC data for these 4 participants were highly correlated, suggesting that the low SAA observed may also indicate a low SAC. The CL/F values for these 4 patients are 136% to 326% of the IM mean CL/F value.
Seventeen ADA positive participants with high SAA levels (≥ 0.1 IU/mL) at matching time points in Course 1 were observed, suggesting that a low SAA may not be due to a positive ADA.

Based on observed IV data, 3 ADA positive participants with lower SAA levels (< 0.1 IU/mL) at matching time points in Course 1 were observed (Table 7). The SAA and SAC data for these 3 participants are highly correlated, suggesting that the low SAA observed may be due to low SAC. Four ADA positive participants with high SAA levels (≥ 0.1 IU/mL) at matching time points in Course 1 were observed, suggesting that a low SAA may not be due to a positive ADA.

2.5.4. Conclusions on clinical pharmacology

The applicant has adequately characterised the pharmacokinetic and pharmacodynamic properties of recombinant crisantaspase produced in *Pseudomonas fluorescens*.

2.5.5. Clinical efficacy

2.5.5.1. Dose response study

The proposed dose is based on data from participants in the investigational cohorts in the ongoing pivotal Phase 2/3 Study JZP458-201, and Phase 1 Study JZP458-101 in healthy adult participants and popPK modeling and simulation, which are described in the PK section of this report.

2.5.5.2. Main study

JZP458-201: A phase 2/3 open-label, multi-center study of JZP-458 in participants with ALL/LBL following hypersensitivity of E. coli-derived asparaginases.

Methods

The study consisted of two parts and multiple sub-cohorts investigating recombinant crisantaspase produced in Pseudomonas fluorescens (RC-P) at different dose levels and administration routes:

- Part A investigating intramuscular (**IM**) RC-P
    - Cohort 1 (dose-finding)
        - In Cohort 1a, an IM dose of 25 mg/m^2 was administered on a Monday, Wednesday, Friday (MWF) schedule (i.e., 25/25/25).
        - In Cohort 1b, an IM dose of 37.5 mg/m^2 was administered on a MWF schedule (i.e., 37.5/37.5/37.5)
        - Cohort 2, an expansion cohort at the 37.5 mg/m2 dose level.
        - In Cohort 1c, an IM dose of 25 mg/m^2 was administered on Mondays and Wednesdays and 50 mg/m^{2}on Fridays (i.e., 25/25/50).
- Part B investigating intravenous (**IV**) RC-P

- **Study Participants**

Key inclusion criteria

- Paediatric and adult patients with a diagnosis of ALL or LBL.
- Cohort 1 (Part A and Part B): Have had a ≥ Grade 3 allergic reaction to a long-acting E. coli-derived asparaginase OR have silent inactivation. Cohort 2 ONLY (Part A): Have had a ≥ Grade 2 allergic reaction to a long-acting E. coli-derived asparaginase OR have silent inactivation. For patients with a Grade 2 allergic reaction, SAA testing for inactivation is strongly encouraged. For the purposes of this study, the definition of silent inactivation is as follows: patients with documented NSAA levels as described below at one of the following time points after

completion of a long-acting E. coli-derived asparaginase infusion (but without clinical signs/symptoms of hypersensitivity or allergic reaction):

  - Time point of 1 hour to 1 day with NSAA < 0.5 IU/mL
  - Time point of ≤ 7 days with NSAA < 0.3 IU/mL
  - Time point of≤14 days with NSAA < 0.1 IU/mL

- Have 1 or more courses of E. coli-derived asparaginase (ie, to allow for at least 6 doses of RC-P) remaining in his/her treatment plan.
- Patients must have, in the opinion of the Investigator, fully recovered from their prior allergic reaction to E. coli-derived asparaginase. Patients must have completed antihistamine, epinephrine, and/or corticosteroid treatment for the allergic reaction ≥ 24 hours prior to RC-P administration. Undetectable SAA levels (defined as ≤ 0.02 IU/mL) must also be documented prior to enrolment in the study, except for patients who received less than 10% of an E. coli-derived asparaginase intravenous infusion prior to the reaction.
- Have adequate liver function, defined as: direct (conjugated) bilirubin ≤ 3X upper limit of normal (ULN); SGPT (ALT) and SGOT (AST) ≤ 5X ULN.
- Female subjects of childbearing potential (ie, fertile, following menarche) and male subjects who have female partners of childbearing potential must agree to use medically acceptable methods of contraception with their partners from Screening, throughout the study, and for 30 days after the last dose of RC-P.

Key exclusion criteria

- Have previously received asparaginase *Erwinia chrysanthemi* or RC-P.
- Have relapsed ALL or LBL.
- Are concurrently receiving another investigational agent and/or treated with an investigational device at the same time as RC-P (within 48 hours) during Course 1 of RC-P.
- Have a history of ≥ Grade 3 pancreatitis.
- Prior history of asparaginase-associated ≥ Grade 3 hemorrhagic event or asparaginase-associated thrombus requiring anticoagulation therapy, excluding catheter-related thrombotic events.
- Patients who have any serious active disease or co-morbid medical condition (according to Investigator's decision), or psychiatric illness that would prevent the patient from signing the informed consent form, assent form or informed consent form by parents, pending institutional requirements, or per Investigator's opinion, would prevent the patient from completing one course of RC-P.

- **Treatments**

Part A

In Cohorts 1a, 1b, and 1c of Part A of the study, RC-P was administered via the IM route, and the volume of JZP-458 at a single injection site is limited to 2 mL with the use of multiple injection sites, as needed.

- In Cohort 1a, an IM dose of 25 mg/m^2 was administered on a MWF schedule (i.e. 25/25/25).

- In Cohort 1b, an IM dose of 37.5 mg/m^2 was administered on a MWF schedule (i.e. 37.5/37.5/37.5)
- In Cohort 1c, an IM dose of 25 mg/m^2 was administered on Mondays and Wednesdays and 50 mg/m2 on Fridays (i.e. 25/25/50).

Cohort 2 was an expansion cohort to confirm the efficacy and safety of the final IM RC-P dose level and schedule.

Part B

In Part B of the study, RC-P was administered via the IV route, with 25 mg/m^2 on Mondays and Wednesdays and as 50 mg/m^2 on Fridays (i.e. 25/25/50).

For both part A and part B of the study, a cycle was defined as 6 injections of RC-P. Dosages (mg/m^2) were administered based on the participant's body surface area (BSA) and calculated using the participant's height and weight measurements taken prior to the start of each course.

All other therapy is being continued according to the therapeutic regimen as defined in the participant's original treatment protocol for the participant's ALL/LBL.

- **Objectives**

The objectives of JZP458-201 are described in ***Table 4***.

- **Outcomes/endpoints**

The endpoints of Study JZP458-201 are described in ***Table 4***.

Table 4. Objectives and endpoints of Study JZP458-201

Primary Efficacy Objective:	Primary Endpoints
• To determine the efficacy of IM JZP-458 administration as measured by the response in Cohort 1 and Cohort 2, defined as the last 72-hour NSAA level ≥ 0.1 IU/mL during the first course.	• The primary efficacy endpoint of the study is the response rate, defined as the proportion of participants with the last 72-hour NSAA level ≥ 0.1 IU/mL during the first course of IM JZP-458. Depending on the JZP-458 start day for a participant, this could be predose 4 if the first course of JZP-458 started on a Monday; predose 6 if the first course of JZP-458 started on a Wednesday; or predose 5 if the first course of JZP-458 started on a Friday.
Key Secondary Objectives	**Key Secondary Endpoints**
• The key secondary objective of the study was to determine the efficacy of IM JZP-458 administration as measured by the response in Cohort 1 and Cohort 2, defined as the last 48-hour NSAA level ≥ 0.1 IU/mL during the first course.	• The proportion of participants with the last 48-hour NSAA level ≥ 0.1 IU/mL during the first course of IM administration of JZP-458.
Secondary Objectives	**Secondary Endpoints**
• To determine the efficacy of IM JZP-458 administration as measured by the response in Cohort 1 and Cohort 2, defined as the last 48-hour and the last 72-hour NSAA levels ≥ 0.4 IU/mL during the first course. • To characterize the PK of IM JZP-458 using a population PK approach, and to explore exposure-response correlations. • To assess the immunogenicity of IM JZP-458 following repeat administration of JZP-458.	• Proportion of participants with the last 48-hour NSAA level ≥ 0.4 IU/mL during the first course of IM administration of JZP-458. • Proportion of participants with the last 72-hour NSAA level ≥ 0.4 IU/mL during the first course of IM administration of JZP-458. • Characterization of the PK of IM JZP-458 based on SAA using a population PK approach and exposure-response correlations. • Incidence of ADA formation against JZP-458.
Exploratory Objectives	**Exploratory Endpoints**
• To determine the efficacy of IV JZP-458 administration as measured by the response, defined as the last 48-hour NSAA level ≥ 0.1 IU/mL and the last 72-hour NSAA level ≥ 0.1 IU/mL during the first course.	• Proportion of participants with the last 48-hour NSAA ≥ 0.1 IU/mL during the first course of IV administration of JZP-458. • Proportion of participants with the last 72-hour NSAA ≥ 0.1 IU/mL during the first course of IV administration of JZP-458.
• To determine the efficacy of IV JZP-458 administration as measured by the response, defined as the last 48-hour NSAA level ≥ 0.4 IU/mL and the last 72-hour NSAA level ≥ 0.4 IU/mL during the first course.	• Proportion of participants with the last 48-hour NSAA ≥ 0.4 IU/mL during the first course of IV administration of JZP-458. • Proportion of participants with the last 72-hour NSAA ≥ 0.4 IU/mL during the first course of IV administration of JZP-458.
• To characterize the PK of IV JZP-458 using a population PK approach.	• Characterization of the PK of IV JZP-458 based on SAA using a population PK approach.
• To assess the immunogenicity of IV JZP-458 following repeat administration of JZP-458.	• Incidence of anti-drug antibody formation against JZP-458.

Abbreviations: ADA = anti-JZP-458 antibodies; IM = intramuscular injection; IV = intravenous; NSAA = nadir serum asparaginase activity; JZP-458 = recombinant crisantaspase produced in *Pseudomonas fluorescens;* PK = pharmacokinetics; SAA = serum asparaginase activity.
Note: The Efficacy Analysis Set includes all participants who received at least 1 dose of JZP-458 and had at least one 48- or 72-hour NSAA assessment collected within the protocol-defined sample collection window (±2 hours) in Course 1.

- **Sample size**

Part A

For the final IM RC-P dose level, 13 evaluable patients were planned in Part A Cohort 1, and approximately 85 patients are planned in Cohort 2 to obtain 98 patients in total at the final dose in Part A for the primary efficacy analysis of the IM administration route.

The sample size of 13 evaluable patients in Part A Cohort 1 was to provide at least 80% posterior probability of the true response rate ≥ 96% given 100% response rate in Cohort 1 and noninformative neutral beta prior with $\alpha = \beta = 1/3$. Since the primary efficacy endpoint was considered to be met if the lower bound of the 95% Wald confidence interval (CI) of the response rate exceeds 90%, the final sample size was planned as 98 patients, which would provide 83% probability that the lower bound of the 95% Wald CI exceeds 90%, assuming a true response rate of 96% for the primary efficacy endpoint and a 5% drop out rate.

Additional patients were planned to be enrolled, if fewer than 93 out of 98 patients have the necessary data to be included in the Efficacy Analysis Set.

Interim analysis

One interim analysis with 51 patients was planned. At the interim analysis, a sample size of 51 patients would provide 70% probability that the lower bound of the 95% CI exceeds 90% under the assumption of a 96% true response rate and a 5% drop out rate. Additional patients were planned to be enrolled, if fewer than 48 out of 51 patients have the necessary data to be included in the Efficacy Analysis Set.

Part B

A target of at least 6 evaluable patients were to be administered the first IV dose level. This sample size was not based on formal power calculation but provides an initial assessment of the IV RC-P dosing.

- **Randomisation and blinding (masking)**

This was a single arm, open-label study.

- **Statistical methods**

Efficacy analysis

The primary and key secondary efficacy endpoint will be estimated using the Efficacy Analysis Set for patients administered the final IM RC-P dose level with at least one 72-hour (48 hour for key secondary endpoint) NSAA assessment collected within the protocol defined sample collection window (± 2 hours) in Course 1 of Part A. The last observed 72-hour (/48 hour, respectively) NSAA assessment collected within the protocol defined sample collection window (± 2 hours) in Course 1 will be used in the calculation of the two endpoints. The response rate, along with the 95% Wald CI will be provided. The primary and key secondary efficacy endpoint were to be considered met if the lower bounds of the 95% CI of the response rates exceeded 90%.

Multiplicity control

No formal statistical testing was planned for this study and no multiplicity adjustment was applicable.

Interim analysis

One interim analysis was planned after the 51st patient completes Course 1 with the final IM RC-P dose level in part A (Cohort 1 and Cohort 2 patients). It was planned to stop enrollment of this cohort early for efficacy, in case the primary endpoint was met at the interim analysis. The SAP also states that if the incidence of allergic reactions (including hypersensitivity and anaphylaxis) related to RC-P exceeds 25%, or the incidence of pancreatitis exceeds 10% or thrombosis exceeds 10% at this interim analysis, enrollment into this Expansion Cohort may be stopped for safety.

One primary analysis is planned after the 98th patient completes Course 1 at the final IM RC-P dose in Part A (Cohorts 1 and 2), and

The final analysis was planned when the overall study (Part A and Part B) is completed and all enrolled patients have completed all of their planned courses of RC-P, including end of study procedures, or have discontinued the study early.

Handling of missing values/censoring/discontinuations

For the primary and key secondary endpoints, missing data will not be imputed.

Results

- **Participant flow**

In total 167 patients were enrolled and treated in part A, of whom 51 at the intended IM dose level in Cohort 1c (25/25/50). A total of 62 patients was enrolled in IV part B.

As of the final data cut-off date of 22 Nov 2022, the median (range) number of courses completed was 5 (0, 15) in part A and 3 (0, 15) in part B. In IM Part A, 77.2% of patients completed all planned courses (**Table *5*)**. In IV Part B, this applied to 43.5% of the patients.

Table 5. Disposition of patients in study JZP458-201 (Final data cut-off Nov 2022)

	IM 25 mg/m² (MWF) (N = 33)	IM 37.5 mg/m² (MWF) (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 62)	IV Total (N = 62)
Participants screened, n	–	–	–	174	–	64
Participants who screen failed, n **Reason for screen failure**	–	–	–	7	–	2
Inclusion/exclusion criteria not met	–	–	–	2	–	0
Withdrawal of consent	–	–	–	4	–	0
Death	–	–	–	0	–	0
Lost to follow-up	–	–	–	0	–	0
Other	–	–	–	1	–	2
Participants in the Enrolled Analysis Set, n	33	83	51	167	62	62
Participants received at least 1 dose of JZP-458 treatment, n (%)	33 (100)	83 (100)	51 (100)	167 (100)	61 (98.4)	61 (98.4)
Participants completed all planned JZP-458 treatment, n (%)	27 (81.8)	62 (74.7)	40 (78.4)	129 (77.2)	27 (43.5)	27 (43.5)
Participants discontinued JZP-458 treatment, n (%) **Reason for discontinuing JZP-458 treatment**	6 (18.2)	21 (25.3)	11 (21.6)	38 (22.8)	34 (54.8)	34 (54.8)
Adverse event, n (%)	3 (9.1)	14 (16.9)	6 (11.8)	23 (13.8)	20 (32.3)	20 (32.3)
Death, n (%)	0	0	0	0	0	0
Lost to follow-up, n (%)	0	0	0	0	0	0
Physician decision, n (%)	2 (6.1)	5 (6.0)	2 (3.9)	9 (5.4)	7 (11.3)	7 (11.3)
Pregnancy, n (%)	0	0	0	0	0	0
Progressive disease, n (%)	0	2 (2.4)	1 (2.0)	3 (1.8)	1 (1.6)	1 (1.6)
Protocol deviation, n (%)	0	0	0	0	0	0
Recurrent disease, n (%)	0	0	0	0	1 (1.6)	1 (1.6)
Study terminated by sponsor, n (%)	0	0	0	0	0	0
Study site terminated by sponsor, n (%)	0	0	0	0	0	0
Withdrawal by parent or guardian, n (%)	1 (3.0)	0	0	1 (0.6)	3 (4.8)	3 (4.8)
Withdrawal by participant, n (%)	0	0	1 (2.0)	1 (0.6)	1 (1.6)	1 (1.6)
Other, n (%)	0	0	1 (2.0)	1 (0.6)	1 (1.6)	1 (1.6)
Participants completed study, n (%)	27 (81.8)	62 (74.7)	39 (76.5)	128 (76.6)	27 (43.5)	27 (43.5)
Participants discontinued study, n (%) **Reason for discontinuing study**	6 (18.2)	21 (25.3)	12 (23.5)	39 (23.4)	35 (56.5)	35 (56.5)
Adverse event, n (%)	2 (6.1)	12 (14.5)	6 (11.8)	20 (12.0)	21 (33.9)	21 (33.9)
Death, n (%)	1 (3.0)	2 (2.4)	0	3 (1.8)	0	0
Lost To follow-up, n (%)	0	0	0	0	0	0
Physician decision, n (%)	2 (6.1)	5 (6.0)	2 (3.9)	9 (5.4)	7 (11.3)	7 (11.3)
Pregnancy, n (%)	0	0	0	0	0	0
Progressive disease, n (%)	0	2 (2.4)	1 (2.0)	3 (1.8)	1 (1.6)	1 (1.6)
Protocol deviation, n (%)	0	0	1 (2.0)	1 (0.6)	0	0
Recurrent disease, n (%)	0	0	0	0	1 (1.6)	1 (1.6)
Study terminated by sponsor, n (%)	0	0	0	0	0	0
Study site terminated by sponsor, n (%)	0	0	0	0	0	0
Withdrawal by parent or guardian, n (%)	1 (3.0)	0	0	1 (0.6)	3 (4.8)	3 (4.8)
Withdrawal by participant, n (%)	0	0	1 (2.0)	1 (0.6)	1 (1.6)	1 (1.6)
Other, n (%)	0	0	1 (2.0)	1 (0.6)	1 (1.6)	1 (1.6)

Abbreviations: F = Friday; IM = intramuscular; IV = intravenous; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday.
Percentages were calculated with the number of participants in the Enrolled Analysis Set as the denominator.

- **Recruitment**

The first patient signed informed consent on 27 December 2019. The data cut-off data for the interim analysis was 19 July 2021. The data cut-off for the final analysis was 22 November 2022.

- **Conduct of the study**

Protocol amendments

The original protocol was approved on 29 Aug 2019 and consequently amended twice. A summary of key changes is provided below.

Protocol amendment 1 – Aug 2020

- To allow testing of additional subcohorts at higher doses above 37.5 mg/m^2, with a maximum of 80 mg/m^2.
- To allow enrollment into Part B (IV) to begin before completion of part A (IM).
- To allow the SDRC to recommend a different dose to be given on Fridays than on Mondays or Wednesdays, to ensure adequate coverage for up to 72 hours.

Protocol amendment 2 – Sep 2020

- The dose capp of 80 mg/m^2 was removed for additional subcohorts, as preliminary PK modeling data had shown that higher doses may be necessary to achieve the objectives.

Changes to planned analyses

The statistical analysis plan (SAP) was amended 4 times. These mostly pertained to clarifications, addition of COVID-19 sensitivity analyses and update of study design following the protocol amendments.

Protocol deviations

Overall, 27.5% of patients in part A and 37.7% of patients in part B of the study had 1 or more major protocol deviations at the final analysis data cut-off. In part A, the most frequently reported deviation was laboratory deviation (28 of 167 [16.8%] participants). In part B, the most frequently reported major protocol deviation was study procedures deviation (13 of 61 [21.3%] participants).

- **Baseline data**

Demographic and baseline characteristics in in study JZP458-201 are summarised in**Table *6***.

Table 6.Demographic and baseline characteristics (Safety Analysis Set 19 July 2021)

	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Sex (n [%])					
Female	16 (48.5)	28 (33.7)	20 (39.2)	64 (38.3)	25 (41.0)
Male	17 (51.5)	55 (66.3)	31 (60.8)	103 (61.7)	36 (59.0)
Declined to state	0	0	0	0	0
Ethnicity (n [%])					
Hispanic or Latino	13 (39.4)	23 (27.7)	17 (33.3)	53 (31.7)	20 (32.8)
Not Hispanic or Latino	18 (54.5)	56 (67.5)	32 (62.7)	106 (63.5)	35 (57.4)
Declined to state	2 (6.1)	4 (4.8)	2 (3.9)	8 (4.8)	6 (9.8)
Race (n [%])					
American Indian or Alaska Native	0	0	3 (5.9)	3 (1.8)	2 (3.3)
Asian	1 (3.0)	5 (6.0)	1 (2.0)	7 (4.2)	3 (4.9)
Black or African American	3 (9.1)	11 (13.3)	8 (15.7)	22 (13.2)	2 (3.3)
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
White	24 (72.7)	58 (69.9)	33 (64.7)	115 (68.9)	43 (70.5)
Declined to State	0	0	0	0	0
Multiple	1 (3.0)	0	0	1 (0.6)	1 (1.6)
Not Reported	4 (12.1)	9 (10.8)	6 (11.8)	19 (11.4)	10 (16.4)
Age at enrollment (years)					
n	33	83	51	167	61
Mean (SD)	11.5 (7.11)	9.0 (5.08)	11.3 (5.41)	10.2 (5.71)	10.4 (6.30)
Median	11.0	8.0	12.0	10.0	10.0
Minimum, Maximum	1, 24	1, 20	3, 25	1, 25	1, 24
Age subgrouping (n [%])					
< 6 years	9 (27.3)	24 (28.9)	11 (21.6)	44 (26.3)	20 (32.8)
6 years to < 12 years	9 (27.3)	34 (41.0)	14 (27.5)	57 (34.1)	14 (23.0)
12 years to < 18 years	7 (21.2)	20 (24.1)	18 (35.3)	45 (26.9)	17 (27.9)
≥ 18 years	8 (24.2)	5 (6.0)	8 (15.7)	21 (12.6)	10 (16.4)
< 1 year	0	0	0	0	0
1 year to < 6 years	9 (27.3)	24 (28.9)	11 (21.6)	44 (26.3)	20 (32.8)
6 years to < 12 years	9 (27.3)	34 (41.0)	14 (27.5)	57 (34.1)	14 (23.0)
12 years to < 17 years	6 (18.2)	15 (18.1)	17 (33.3)	38 (22.8)	16 (26.2)

	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
≥ 17 years	9 (27.3)	10 (12.0)	9 (17.6)	28 (16.8)	11 (18.0)
Body surface area (m²) (n [%])					
n	33	82	51	166	61
Mean (SD)	1.283 (0.540)	1.149 (0.453)	1.346 (0.530)	1.236 (0.500)	1.256 (0.556)
Median	1.280	1.010	1.290	1.185	1.180
Minimum, Maximum	0.44, 2.53	0.56, 2.26	0.54, 2.43	0.44, 2.53	0.52, 2.42
Body surface area (n [%])					
0 < BSA ≤ 1	12 (36.4)	41 (49.4)	17 (33.3)	70 (41.9)	26 (42.6)
1 < BSA ≤ 2	19 (57.6)	36 (43.4)	28 (54.9)	83 (49.7)	30 (49.2)
2 < BSA	2 (6.1)	5 (6.0)	6 (11.8)	13 (7.8)	5 (8.2)
Primary disease (n [%])					
ALL	0	0	0	0	0
B-ALL	27 (81.8)	60 (72.3)	37 (72.5)	124 (74.3)	51 (83.6)
T-ALL	4 (12.1)	13 (15.7)	9 (17.6)	26 (15.6)	7 (11.5)
LBL	0	0	0	0	0
B-LBL	0	0	1 (2.0)	1 (0.6)	2 (3.3)
T-LBL	2 (6.1)	10 (12.0)	4 (7.8)	16 (9.6)	1 (1.6)
Time since primary disease diagnosis to Study Day 1 (n [%])					
0 to 3 months	28 (84.8)	55 (66.3)	39 (76.5)	122 (73.1)	47 (77.0)
4 to 6 months	5 (15.2)	24 (28.9)	11 (21.6)	40 (24.0)	14 (23.0)
7 to 9 months	0	4 (4.8)	1 (2.0)	5 (3.0)	0
10 to 12 months	0	0	0	0	0
> 12 months	0	0	0	0	0
Prior asparaginase treatment (n [%])					
Oncaspar	33 (100)	83 (100)	51 (100)	167 (100)	60 (98.4)
Calaspagase Pegol-mknl (Asparlas)	0	0	0	0	0

	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Erwinia Chrysanthemi L-asparaginase	0	0	0	0	0
Other	0	0	0	0	1 (1.6)
Time since last asparaginase received to Study Day 1 (days)					
n	33	83	51	167	61
Mean (SD)	16.8 (20.90)	27.6 (37.67)	23.8 (29.73)	24.3 (32.68)	24.9 (28.98)
Median	9.0	10.0	10.0	10.0	13.0
Minimum, Maximum	2, 116	2, 158	2, 133	2, 158	2, 138
Eligibility Criteria (n [%])					
Grade 2 allergic reaction to an *E. coli*-derived asparaginase	0	0	0	0	0
Grade ≥ 3 allergic reaction to an *E. coli*-derived asparaginase	27 (81.8)	75 (90.4)	44 (86.3)	146 (87.4)	44 (72.1)
Silent inactivation	3 (9.1)	3 (3.6)	1 (2.0)	7 (4.2)	8 (13.1)
Allergic reaction with inactivation	3 (9.1)	5 (6.0)	6 (11.8)	14 (8.4)	9 (14.8)

Abbreviations: ALL = acute lymphoblastic leukemia; B-ALL = B-cell acute lymphoblastic leukemia; B-LBL = B-cell lymphoblastic lymphoma; BSA = body surface area; *Escherichia coli* = *E. coli*; IM = intramuscular; IV = intravenous; LBL = lymphoblastic lymphoma; MWF = Monday, Wednesday, Friday; T-ALL = T-cell acute lymphoblastic leukemia; T-cell lymphoblastic lymphoma; T-LBL = T-cell lymphoblastic lymphoma
Percentages were calculated with the number of participants in the Safety Analysis Set as a denominator.
Time since diagnosis in months is calculated by taking the integer of '[(Study Day 1 - Date of Diagnosis + 1)/365.25] × 12'.
A baseline value is defined as the latest non-missing value obtained prior to or at the start date and/or time of the first dose of JZP-458 (Study Day 1).
Study Day 1 is defined as the date of the first dose of JZP-458.

- **Numbers analysed**

The number of participants in each analysis set is shown in **Table *7***.

Table 7. Analysis sets in study JZP458-201

	IM 25 mg/m² MWF	IM 37.5 mg/m² MWF	IM 25 (MW)/ 50 (F) mg/m²	IM Total	IV 25 (MW)/ 50 (F) mg/m²
Participants in the Enrolled Analysis Set, n	33	83	51	167	62
Participants in the Safety Analysis Set, n (%)	33 (100)	83 (100)	51 (100)	167 (100)	61 (98.4)
Participants in the Efficacy Analysis Set, n (%)	32 (97.0)	83 (100)	50 (98.0)	165 (98.8)	59 (95.2)
Participants in the PK Analysis Set, n (%)	32 (97.0)	83 (100)	51 (100)	166 (99.4)	60 (96.8)
Participants in the PD Analysis Set, n (%)	32 (97.0)	83 (100)	51 (100)	166 (99.4)	60 (96.8)

Abbreviations: F = Friday; IM = intramuscular; IV = intravenous; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; NSAA = nadir serum asparaginase activity; PD = pharmacodynamic; PK = pharmacokinetic; SAA = serum asparaginase activity
Percentages were calculated with the number of participants in the Enrolled Analysis Set as a denominator.
The Enrolled Analysis Set includes participants who signed the informed consent and met inclusion/exclusion criteria per the investigator.
The Safety Analysis Set included participants who received at least 1 dose of JZP-458.
The Efficacy Analysis Set included participants who received at least 1 dose of JZP-458 and had at least one 48- or 72-hour NSAA assessment collected within the protocol-defined sample collection window (= 2 hours) in Course 1.
The PK Analysis Set included participants who received at least 1 dose of JZP-458 and had at least 1 postdose evaluable SAA or PK concentration value.
The PD Analysis Set included participants who received at least 1 dose of JZP-458 and had at least 1 postdose evaluable L-asparaginase or L-glutamine value.

- **Outcomes and estimation**

Primary endpoint – Response rate (last 72 hour NSAA level ≥ 0.1 IU/mL during first course IM)

When RC-P was administered IM at the intended dose level of 25 (MW)/ 50 (F) mg/m^2, **89.8%** (95% CI: 81.3%, 98.3%) of patients achieved NSAA levels ≥ 0.1 IU/mL at the last 72-hour assessment during the first course of treatment at the interim analysis (44 out of 49 patients). This result as well as results for the secondary endpoints at the intended dose level remained unchanged at the final analysis (**Table *8***).

Table 8. Proportion of participants with last 72- and 48-hour nadir serum asparaginase activity levels ≥ 0.1 or ⩾ 0.4 IU/mL during the first course of RC-P (Efficacy Analysis Set, final analysis)

NSAA Level	Time Point	IM 25 mg/m² MWF			IM 37.5 mg/m² MWF			IM 25 (MW)/ 50 (F) mg/m²			IV 25 (MW)/ 50 (F) mg/m²		
		N	n (%)	95% CI	N	n (%)	95% CI	N	n (%)	95% CI	N	n (%)	95% CI
≥0.1 IU/mL	Last 48 hour	32	31 (96.9)	90.8, 100.0	83	82 (98.8)	96.4, 100.0	49	47 (95.9)	90.4, 100.0	59	53 (89.8)	82.1, 97.5
	Last 72 hour	28	18 (64.3)	46.5, 82.0	77	70 (90.9)	84.5, 97.3	49	44 (89.8)	81.3, 98.3	50	20 (40.0)	26.4, 53.6
≥0.4 IU/mL	Last 48 hour	32	16 (50.0)	32.7, 67.3	83	65 (78.3)	69.4, 87.2	49	32 (65.3)	52.0, 78.6	59	10 (16.9)	7.4, 26.5
	Last 72 hour	28	1 (3.6)	0.0, 10.4	77	20 (26.0)	16.2, 35.8	49	23 (46.9)	33.0, 60.9	50	0	-

Abbreviations: CI = confidence interval; F = Friday; IM = intramuscular; IV = intravenous; JZP-458 = recombinant crisantaspase *Pseudomonas fluorescens*, also referred to as RC-P; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; NSAA = nadir serum asparaginase activity.
Percentages were calculated with the number of participants for each course and schedule as the denominator.
The Efficacy Analysis Set at 48 hours (ie, key secondary efficacy endpoint) included participants who received at least 1 dose of JZP-458 with at least one 48-hour NSAA assessment collected within the protocol-defined sample collection window (± 2 hours) in Course 1.
The Efficacy Analysis Set at 72 hours (ie, primary efficacy endpoint) included participants who received at least 1 dose of JZP-458 with at least one 72-hour NSAA assessment collected within the protocol-defined sample collection window (± 2 hours) in Course 1.
The 95% CI was calculated by the Wald method.

Key secondary endpoint - Response rate (last 48 hour NSAA level ≥ 0.1 IU/mL during first course IM)

When RC-P was administered IM at doses of 25 (MW)/ 50 (F) mg/m^2, 95.9% (95% CI: 90.4%, 100.0%) of patients achieved NSAA levels ≥ 0.1 IU/mL at the last 48-hour assessment during the first course of treatment at the interim as well as final analysis (47 of 49 patients, **Table 14**).

Other secondary endpoints

Response rate (with last 72 hour NSAA level ≥ 0.4 IU/mL during first course IM)

When RC-P was administered IM at doses of 25 (MW)/ 50 (F) mg/m^2, 46.9% (95% CI: 33.0%, 60.9%) of patients achieved NSAA levels ≥ 0.4 IU/mL at the last 72-hour assessments, respectively, during the first course of treatment (23 of 49 patients).

Response rate (with last 48 hour NSAA level ≥ 0.4 IU/mL during first course IM)

When JZP-458 was administered IM at doses of 25 (MW)/ 50 (F) mg/m^2, 65.3% (95% CI: 52.0%, 78.6%) of participants achieved NSAA levels ≥ 0.4 IU/mL at the last 48-hour assessments, respectively, during the first course of treatment (32 of 49 patients).

Exploratory endpoints (IV administration)

Response rate (with last 48 hour NSAA level ≥ 0.4 IU/mL during first course IV)

At RC-P IV dose levels administered IV at doses of 25 (MW)/ 50 (F) mg/m^2, 16.9% (95% CI: 7.4%, 26.5%) of patients achieved NSAA levels ≥ 0.4 IU/mL at the last 48-hour assessment during the first course of treatment.

At RC-P IV dose levels administered IV at doses of 25 (MW)/ 50 (F) mg/m^2, 89.8% (95% CI: 82.1%, 97.5%) of patients achieved NSAA levels ≥ 0.1 IU/mL at the last 48-hour assessment during the first course of treatment.*Response rate (with last 72 hour NSAA level ≥ 0.4 IU/mL during first course IV).*

At RC-P dose levels administered IV at doses of 25 (MW)/ 50 (F) mg/m^2, no patients achieved NSAA levels ≥ 0.4 IU/mL at the last 72-hour assessment during the first course of treatment.

At RC-P IV dose levels 25 (MW)/ 50 (F) mg/m^2 administered on a MWF schedule, 40.0% (95% CI: 26.4%, 53.6%) of patients, respectively, achieved NSAA levels ≥ 0.1 IU/mL at the last 72-hour assessment during the first course of treatment.

In line with the intended IM dose level Cohort C, results were consistent between the interim and final analysis for IV Part B of the study as well.

Ancillary analyses

Treatment effect beyond course 1

The applicant presented therapeutic drug monitoring (TDM) data for all courses in Part A and B (**Table *9*, Figure 2,*Figure 3***). For Cohort c at the targeted IM dose level 25 (MW)/50 (F), the last 48-hr mean SAA level exceeded 0.1 IU/mL (the accepted threshold to demonstrate adequate asparagine depletion in clinical practice) in all 10 courses. For IV Part B last 48-hr mean SAA levels exceeded the threshold in all courses, except for Cycle 7 (0.0810 IU/mL). Mean SAA levels ≥ 0.1 IU/mL at the last 72-hour assessment in IM Part A Cohort C exceeded 0.1 IU/mL in the first 6 courses. In IV Part B last 72-hr mean SAA levels did reach the threshold in cycles 2, 3 and 5.

Table 9. Summary of mean serum asparaginase activity results (IU/mL) with RC-P IM and IV in courses 1 to 10 (Efficacy Analysis Set)

Course Number		Part A - IM 25 (MW)/ 50 (F) mg/m²			Part B - IV 25 (MW)/ 50(F) mg/m²		
		N	Mean (SD) SAA Levels (IU/mL)	95% CI	N	Mean (SD) SAA Levels (IU/mL)	95% CI
1	Last 48-hr	49	0.6550 (0.4121)	0.5366, 0.7733	59	0.2450 (0.1732)	0.1999, 0.2902
	Last 72-hr	49	0.4677 (0.4125)	0.3492, 0.5862	50	0.1016 (0.0986)	0.0736, 0.1297
2	Last 48-hr	42	0.7146 (0.5763)	0.5350, 0.8942	34	0.2664 (0.2230)	0.1886, 0.3442
	Last 72-hr	38	0.5518 (0.5691)	0.3647, 0.7389	32	0.1092 (0.1270)	0.0634, 0.1550
3	Last 48-hr	39	0.6609 (0.4675)	0.5093, 0.8124	26	0.3095 (0.2258)	0.2183, 0.4007
	Last 72-hr	35	0.4447 (0.4050)	0.3056, 0.5838	25	0.1174 (0.1038)	0.0746, 0.1603
4	Last 48-hr	36	0.6536 (0.5125)	0.4802, 0.8270	22	0.3487 (0.2594)	0.2337, 0.4637
	Last 72-hr	31	0.4739 (0.5024)	0.2896, 0.6582	17	0.1076 (0.0893	0.0618, 0.1535
5	Last 48-hr	24	0.6645 (0.5342)	0.4389, 0.8900	18	0.5996 (1.1647)	0.0204, 1.1788
	Last 72-hr	26	0.4510 (0.4856)	0.2548, 0.6471	18	0.1339 (0.1170)	0.0757, 0.1921
6	Last 48-hr	16	0.7065 (0.4906)	0.4450, 0.9679	12	0.2768 (0.2253)	0.1337, 0.4200
	Last 72-hr	18	0.4787 (0.4070)	0.2763, 0.6811	11	0.0885 (0.0771)	0.0367, 0.1403
7	Last 48-hr	2	0.1518 (0.0385)	0, 0.4980	3	0.0810 (0.0539)	0, 0.2150
	Last 72-hr	2	0.0396 (0.0560)	0, 0.5428	3	0.0601 (0.0117)	0.0309, 0.0893
8	Last 48-hr	2	0.1376 (0.0278)	0, 0.3872	3	0.1469 (0.0676)	0, 0.3149
	Last 72-hr	2	0.0694 (0.0163)	0, 0.2161	3	0.0669 (0.0753)	0, 0.2539
9	Last 48-hr	1	0.4295 (N/A)	N/A	3	0.1052 (0.0476)	0, 0.2234
	Last 72-hr	1	0.2358 (N/A)	N/A	3	0.0458 (0.0070)	0.0284, 0.0633
10	Last 48-hr	1	0.3852 (N/A)	N/A	3	0.1165 (0.1174)	0, 0.4081
	Last 72-hr	1	0.0638 (N/A)	N/A	3	0.0477 (0.0420)	0, 0.1521

Abbreviations: CI = confidence interval; F = Friday; IM = intramuscular; IV = intravenous; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; NSAA = Nadir serum asparaginase activity; SAA = serum asparaginase activity; SD = standard deviation.
The Efficacy Analysis Set included participants who received at least 1 dose of JZP-458 with at least one 48-hour or 72-hour NSAA assessment collected within the protocol-defined sample collection window (± 2 hours) in Course 1.
If mean < lower limit of quantitation, then SD and CI were not calculated.

***Figure 2. Mean (95% CI) of last 48-hour and last 72-hour serum asparaginase activity with IM RC-P in courses 1 to* 10 (Efficacy Analysis Set).**

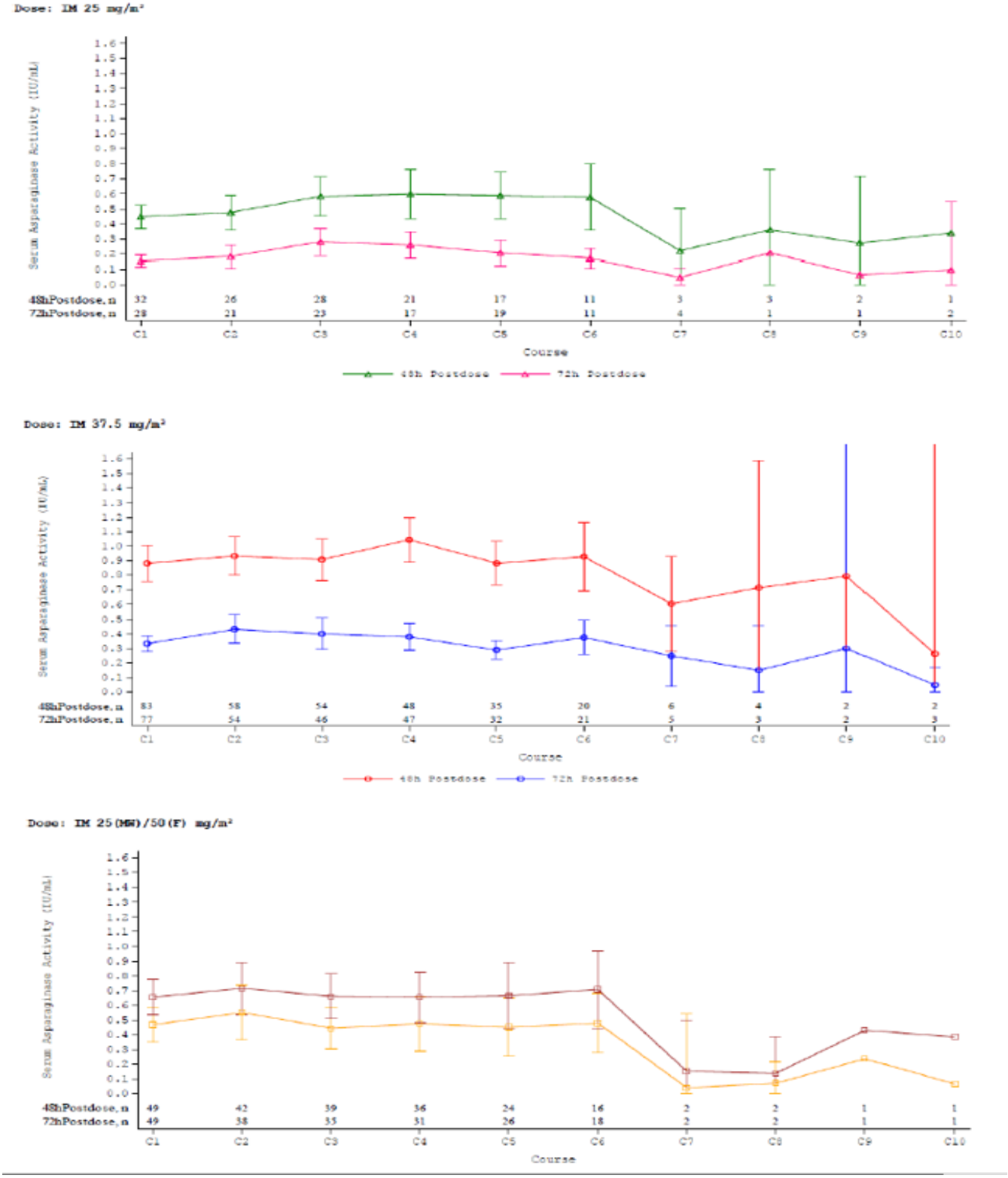
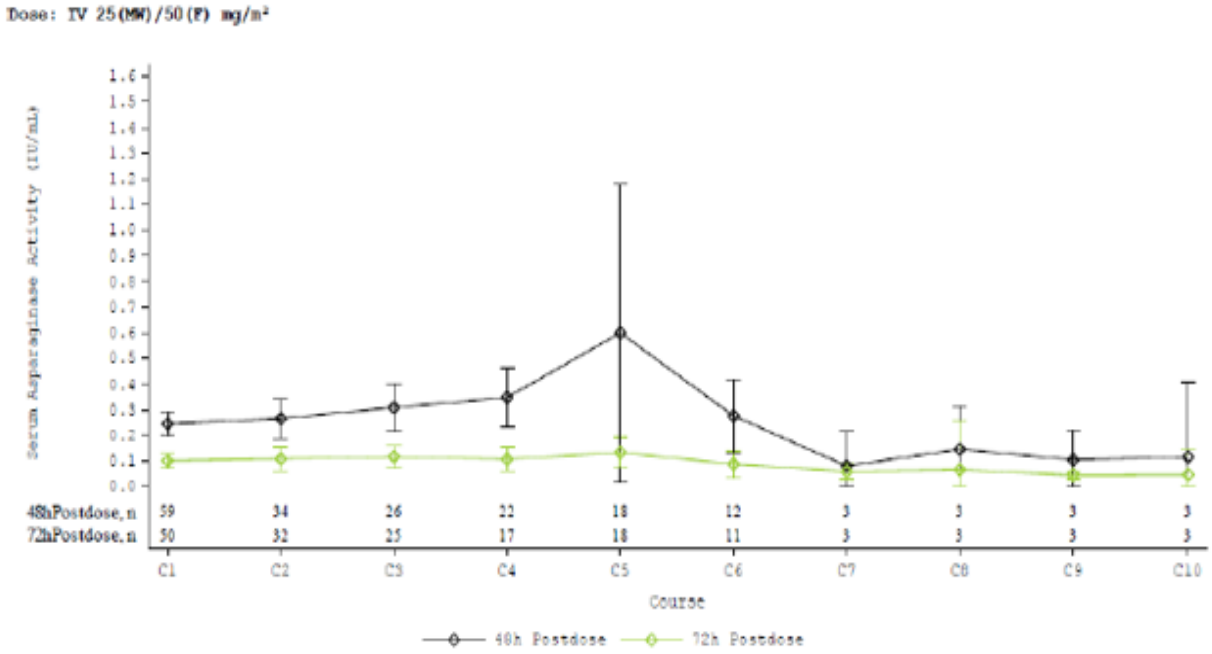


Figure 3. Mean (95% CI) of last 48-hour and last 72-hour serum asparaginase activity with IV RC-P in courses 1 to 10 (Efficacy Analysis Set).



- **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 10. Summary of efficacy for trial JZP458-201

Title: An Open-label, Multicenter Study of RC-P in Patients With Acute Lymphoblastic Leukemia (ALL)/Lymphoblastic Lymphoma (LBL) Following Hypersensitivity to E. coli-derived Asparaginases			
Study identifier	Study number: JZP458-201 NCT number: NCT04145531		
Design	Open-label, multicenter, single-arm, dose confirmation and pharmacokinetic (PK) study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Start 27 Dec 2019, Interim data cut-off 19 Jul 2021, Final data cut-off 22 Nov 2022 Not applicable
Hypothesis	According to protocol, the primary and key secondary efficacy endpoint will be met if the lower bounds of the 95% CI of the response rates exceed 90%.		
Treatments groups	Efficacy analysis set		IM total: n=165 IM 25/25/50: n=51 *IV: n=59 (exploratory*)*
Endpoints and definitions	Primary endpoint	Response rate last 72-hour NSAA level ≥0.1 IU/mL	Proportion of patients with the last 72-hour NSAA level ≥0.1 IU/mL during the first course of **IM** RC-P. Depending on the start day this could be predose 4 (if first course started on Monday), predose 6 (if started on Wednesday) or predose 5 (if started on Friday).

Title: An Open-label, Multicenter Study of RC-P in Patients With Acute Lymphoblastic Leukemia (ALL)/Lymphoblastic Lymphoma (LBL) Following Hypersensitivity to E. coli-derived Asparaginases			
Study identifier	Study number: JZP458-201 NCT number: NCT04145531		
	Key secondary endpoint	Response rate last 48-hour NSAA level ≥0.1 IU/mL	Proportion of patients with the last 48-hour NSAA level ≥0.1 IU/mL during the first course of **IM** RC-P.
	Other secondary endpoints	Response rate last 72-hour NSAA level ≥0.4 IU/mL Response rate last 48-hour NSAA level ≥0.4 IU/mL	Proportion of patients with the last 72-hour NSAA level ≥0.4 IU/mL during the first course of **IM** RC-P. Proportion of patients with the last 48-hour NSAA level ≥0.4 IU/mL during the first course of **IM** RC-P.
Database lock	22 November 2022 (final analysis)		

Results and Analysis

Analysis description	**Interim Analysis**		
Analysis population and time point description	Efficacy analysis set, including patients who signed informed consent and met inclusion/exclusion criteria per investigator (ITT).		
Descriptive statistics and estimate variability	Treatment group	**IM** 25/50/50	***IV*** *25/50/50**
	Number of subject	n=51 (n=49 evaluated)	*n=59*
	Response rate last 72-hour NSAA level ≥0.1 IU/mL 95% CI	89.8% (n=44/49) (81.3, 98.3)	*40% (n=20/50)* *(26.4, 53.6)*
	Response rate last 48-hour NSAA level ≥0.1 IU/mL 95% CI	95.9% (n=47/49) (90.4, 100.0)	*89.9% (n=53/59)* *(52.1, 97.5)*
	Response rate last 72-hour NSAA level ≥0.4 IU/mL 95% CI	46.9% (n=23/49) (33.0, 60.9)	*0 (n=0/50)* *(-,-)*
	Response rate last 48-hour NSAA level ≥0.4 IU/mL 95% CI	65.3% (n=32/49) (52.0, 78.6)	*16.9% (n=10/59)* *(7.4, 26.5)*
Effect estimate per comparison	Not Applicable.		
Notes	***IV** part B of the study was exploratory, please refer to result section above for details on study design.		

2.5.5.3. Clinical studies in special populations

Paediatric patients

The pivotal study mostly included paediatric patients. No dose adjustment is required.

Elderly

No clinical efficacy data in elderly is available, as the oldest included patient in the pivotal clinical trial was 25. Based on popPK analyses predicting similar SAA response across age ranges from 1 month to 80 years old, no dose recommendation is proposed for elderly.

Hepatic impairment

No efficacy data were presented for patients with hepatic impairment. Eligible patients for the pivotal study must have adequate liver function, defined as: direct (conjugated) bilirubin $\leq$ 3X upper limit of normal (ULN); SGPT (ALT) and SGOT (AST) ≤5X ULN. No dose adjustment is recommended in the SmPC for patients with prior hepatic impairment, although there are recommendations to withhold treatment with bilirubin increase during treatment.

Renal impairment

No efficacy data were presented for patients with renal impairment. RC-P has not been studied in patients with moderate or severe renal impairment; there is limited data with RC-P in patients with mild renal impairment.

2.5.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Clinical efficacy is claimed based on PD endpoints from pivotal Study JZP458-201, a single arm ongoing Phase 2/3 study in paediatric and adult participants with ALL or LBL, who developed an allergic reaction or silent inactivation to an *E. coli*-derived asparaginase.

A comparative trial versus the authorised *Erwinia* derived asparaginase crisantaspase, the only alternative treatment option, would have been preferred to establish efficacy of recombinant crisantaspase produced in *Pseudomonas fluorescens* (RC-P). However, due to the repeated recent supply shortages experienced with crisantaspase and well-established intermediate endpoint for efficacy (asparaginase activity), the single arm pivotal trial design was accepted by CHMP.

The study consisted of two parts and multiple sub-cohorts investigating RC-P at different dose levels and administration routes. Two of these, i.e., 25/25/50 mg/m^2 IM and 25/25/50 mg/m^2 IV on a Monday Wednesday Friday (MWF) schedule have been investigated in respectively Part A and Part B of the pivotal trial. The other investigated dosing regimens (25 mg/m^2 and 37.5 mg/m^2 IM, both on a MWF schedule) were part of the dose-finding phase of the study and will not be discussed here. The applied posology contains 3 additional potential dosing regimens, i.e., 25 mg/m^2 IM Q48h and 25 mg/m^2 IV Q48h (every 48 hour instead of MWF schedules) and the combined IM and IV MWF schedule 25/25/50 IV/IV/IM mg/m^2. The dosing regimens that are not tested are very similar to the dose regimens that were tested in the clinical studies. Of note, all doses in the new regimens (25 mg/m2 IM, 25 mg/m2 IV, and 50 mg/m2 IM) and dose-intervals in the new regimens (48h or 72 h) are also present in one of the dose regimens that were tested in the clinical studies. Although no exact numbers on response rate can be calculated, from pharmacokinetic principles no (large) differences in exposure and response rate are expected for regimens not tested in clinical studies (please refer to PK

section of this report). Importantly, SAA levels can be monitored in clinical practice. The alternative dosing regimens are therefore considered acceptable.

Eligibility criteria are acceptable and result in a study population which reflects the proposed target indication. However, some subpopulations were not recruited (see further discussion below).

Although additional clinical endpoints (like relapse free survival, CR rate and MRD) would have been welcomed, asparaginase activity is considered a well-established intermediate endpoint for asparagine depletion and as such efficacy. Nadir serum asparaginase activity (NSAA) levels ≥ 0.1 IU/mL is the accepted threshold to demonstrate adequate asparagine depletion in clinical practice. Moreover, the difficulty to interpret clinical efficacy results of a single arm trial with enrollment at different stages of ALL treatment is acknowledged.

The predefined target of success for the primary and key secondary endpoint (lower level of 95% CI exceeding 90%) is endorsed. Although cross-study comparison should be interpreted with caution, this lower bound is in line with, if not slightly higher, than the one observed for IM crisantaspase, Study AALL07P2. Nevertheless, a less strict (but sustained) response rate would have been acceptable as well considering the lack of alternative asparaginase treatment options for the proposed target population. Importantly, SAA levels can be monitored in clinical practice.

Endpoints for the requested alternative IV route of administration were defined as exploratory. Considering the definition/analysis of endpoints in line with the IM route, the outcomes are still considered valuable for determination of efficacy.

For a substantial proportion of patients (26-28%) at least one protocol deviation was reported. These mostly concerned laboratory values. Only 16 of 228 patients (7%) had major deviations related to collection of SAA levels, mostly related to 'out of window' collections which were included in the PPK analysis. The impact on reliability of obtained results is likely limited.

The confidence interval around the estimate for the primary endpoint for the first treatment course is expected to be larger than expressed by the presented 95% CI, as the interim analysis has not been adjusted for multiplicity. However, the lower bound of Wald-confidence intervals corrected using either Pocock (80.4%) or Bonferroni-adjustment (80.1%) were similar to the lower bound of an uncorrected Wald 95%-CI (81.3%).

At the request of the CHMP the applicant adjusted the wording of indication to: Enrylaze is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) in adult and paediatric patients (1 month and older) who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase.

Efficacy data and additional analyses

In total 167 patients were included in part A (of whom n=51 at the proposed IM dose level), and 62 patients in IV part B. The median age was 10 years and the majority of patients in both study parts had experienced an allergic reaction to prior asparaginase treatment (72-90%), and B-ALL as primary disease (>74%). The median number of completed courses was 5 in part A and B at the time of the final analysis. Overall, 68% of participants (156/228) completed treatment as planned. Of the participants who discontinued treatment, the median number of courses missed was 3.

The number of included patients with prior silent inactivation to *E. Coli* derived asparaginase alone is very limited (1 patient at the proposed dosing regimen and n=6 at lower dose levels), while these patients form an important part of the target population. Cross-reactivity of RC-P with other asparaginases has not been studied. However, the number of participants with neutralizing antibodies

(NAb) against RC-P is very small (Part A [IM]: n=4; Part B [IV]: n=2); therefore, regarding the potential impact of NAb on SAA no definitive conclusion could be drawn. Nevertheless, there are no indications that there exists cross-reactivity between prior *E.Coli* neutralizing antibodies (NAbs) and RC-P, as a high proportion of patients achieved sufficient SAA levels, all RC-P all predose anti-drug antibody (ADA) positive patients became at some point ADA negative for RC-P, and SAA levels were not decreased in these patients. Please refer to the safety section of this report for further discussion of NAbs.

Although eligible, patients aged 1 month to <1 year, or >25 years were not included. Treatment with PEG asparaginase and development of hypersensitivity/silent inactivation is expected to take longer than 1 month and there is no reason to assume that Enrylaze would not work in paediatric patients <1 year. Based on similar disease characteristics and treatment in patients 1 month and older, extrapolation of data to younger children aged 1 month to 1 year is acceptable. Although response rates for children between 1 month to <1 year are more uncertain, as adjustments in BSA based dosing are described for Spectrila (<1y) and due to the absence of data in this age range in the current dossier, this is acceptable considering the recommended therapeutic drug monitoring (please also refer to PK section of this report). ALL disease characteristics and treatment are not fully similar between the studied population and (older) adults. However, there is no reason to believe that RC-P will be not effective in adults >25 years of age. There are several publications for other asparaginase products (Oncaspar and nationally registered Erwinase) that indicate efficacy of asparaginase therapy in older patients as well. These have been discussed during registration of these products and resulted in an indication that is not restricted by age. Moreover, large effects of developmental processes in elimination pathways on exposure are not expected for the older age groups. As such, extrapolation to patients aged 1 month to <1 year and >25 years is endorsed. In response to the list of questions, the indication has been reworded to adequately reflect the age limit (1 month and older).

Only 1 patient with hypersensitivity or silent inactivation to other E. coli-derived asparaginases (e.g. short acting asparaginase) than Oncaspar-pegaspargase was included. This is endorsed, as pegasparaginase is now well-established as first choice in frontline therapy and adequately reflected in SmPC section 5.1.

Few patients with LBL were included (n=5 at the proposed dose level). In clinical practice ALL and LBL are considered similar in appearance and behaviour and both require intensive treatment. As such, efficacy in LBL can be based on extrapolation from results in ALL.

Results presented for Cohort 1b at the 37 mg/m^2 IM dose level are based on pooled data from Cohort 1b and expansion Cohort 2. In response to the list of questions, it was clarified that patient characteristics of both cohorts are similar and as such, pooling is acceptable.

IM administration

At the interim analysis, the primary endpoint was <u>not</u> met at the intended dose level 25/25/50 nor at one of the other dose levels, as the lower bound of the 95% CI did not exceed the predefined target of 90%. In total 89.8% (95% CI: **81.3**, 98.3) of participants achieved NSAA levels ≥ 0.1 IU/mL at the last 72-hour assessment during the first course of treatment in Cohort 1c. In response to the list of questions, results of the final analysis were presented at which the primary and secondary endpoints at the proposed IM and IV dose levels remained unchanged compared with the interim analysis. Using a 95%-CI with better coverage (Agresti-Coull or Wilson), the lower bound is 78%.

Importantly, although the predefined lower margin of the 95% CI was not met, the response rate is still considered clinically relevant. Final therapeutic drug monitoring (TDM) data over multiple courses demonstrated sustained SAA levels upon repeated administration. The last 48-hr mean SAA level exceeded 0.1 IU/mL with IM administration in all 10 courses analysed and levels at the last 72-hour

assessment exceeded the threshold in the first 6 cycles. TDM is recommended for all patients which should enable reaching sustained high asparaginase activity in a substantial part of the target population. When administered every 48 hours a trough asparaginase activity measurement should be performed at 48 hours post dose. When dosing on a Monday/Wednesday/Friday schedule, trough serum asparaginase activity should be measured 72 hours after the Friday dose and prior to administration of the following Monday dose. The dosing schedule or route of administration should then be individually adapted.

It appears as if with IM RC-P, a slightly lower proportion of patients had sustained asparaginase activity 48- and 72-hour postdose compared to IM Erwinia asparaginase (NSAA levels ≥0.1 IU/mL were 100% at both timepoints for crisantaspase). This should be interpreted with caution due to the well-known caveats of inter-study comparison as well as the small sample size of crisantaspase studies. As discussed above, results are still considered clinically relevant for the target population lacking stable supply of an available alternative asparaginase treatment option.

The predefined target of success was met for the secondary endpoint. The proportion of patients with last 48- hour NSAA level ≥0.1 IU/mL was 95.9% (95% CI: **90.4**, 100.0) during first course IM, with a better confidence interval (Agresti-Coull, Wilson) this becomes 86%. As expected, a lower proportion of patients reached NSAA levels ≥ 0.4 IU/mL at the last 48- and 72-hour assessments (65.3% and 46.9%, respectively).

IV administration

Although in treatment protocols both the IM and IV administration route are allowed, in Europe most patients receive IV asparaginase treatment. Results for M/W/F IV RC-P indicate lower response rates compared to IM administration, which might be expected based on the lower half life (approximately 8 hours vs. 19 hours with the IM route of administration) and experience with crisantaspase. In Part B of the pivotal trial JZP458-201, 40% (95% CI: 26.4, 53.6) of patients achieved NSAA levels ≥0.1 IU/mL at the last 72-hour assessment and 89.8% (95% CI: 82.1, 97.5) at the last 48-hour assessment during the first course of treatment. These response rates are more or less in line with response rates measured with IV crisantaspase. TDM data upon repeated administration indicate sustained SAA levels over multiple courses at the last 48-hour assessment. At the last 72-hour assessment the threshold was only reached in few cycles. It is acknowledged that SmPC section 4.2 has been updated to include a recommendation to monitor asparaginase levels in all patients. If the targeted asparaginase activity levels have not been achieved, it is proposed to switch to an alternative dosing regimen. In this way, the MWF IV route can still be maintained, and as it is less invasive than IM when intravenous access is already in place to administer other anti-cancer medication, it is a valuable alternative for patients who are able to maintain sufficient asparaginase levels. A warning has been included in SmPC section 4.4 to reflect that SAA levels vary substantially between patients with the IV route of administration and to clarify how the dose should be individually adapted when the target level is not reached.

2.5.7. Conclusions on the clinical efficacy

Efficacy is considered established for Enrylaze in the final agreed indication based on PD endpoints related to asparaginase activity from pivotal Study JZP458-201. Despite the relaxed type I error control and a lower than targeted response rate, reported results are still considered clinically relevant. TDM is recommended for all patients in the SmPC which enables high asparaginase activity to be obtained in a substantial part of the target population, for which no (stable supply of an) alternative asparaginase treatment option exists.

With the IV route of administration on a M/W/F schedule, lower response rates were observed compared to IM administration. This is acceptable considering the recommended therapeutic drug

monitoring and potential to switch to an alternative dosing regimen when insufficient asparaginase activity is achieved with IV administration. Moreover, the IV route of administration would allow to stop infusions immediately in case of acute hypersensitivity reactions and increase patient convenience when they already have a central line.

2.5.8. Clinical safety

2.5.8.1. Patient exposure

At the final database lock date of 22 November 22, a total of 167 patients (33 in Cohort 1a [IM 25 mg/m2 MWF], 83 in Cohort 1b [IM 37.5 mg/m2 MWF], and 51 in Cohort 1c [25 (MW)/50 (F) mg/m2]) were included in the Safety Analysis Set for Part A (IM) of the study. Furthermore, 61 patients were included in the Safety Analysis Set for Part B (IV 25 (MW)/50 (F) mg/m2).

Of the 167 patients in the Safety Analysis Set, 44 (26.3%) were <6 years of age, 57 (34.1%) were 6 to <12 years of age, 45 (26.9%) were 12 to <18 years of age, and 21 (12.6%) were ≥ 18 years of age.

Overall in Part A (IM), the median (range) number of RC-P courses completed was 15.0 (0, 15); overall in Part B (IV), the median (range) number of RC-P courses completed was 3.0 (0, 15).

Overall, 68% of participants (156/228) in the JZP458-201 study completed RC-P treatment as planned. Of the participants who discontinued JZP-458 treatment, the mean (SD) and median number of courses missed was 2.8 (1m7) and 3, respectively.

2.5.8.2. Adverse events

Overall, 164 of 167 patients (98.2%) in Part A (IM) and 60 of 61 (98.4%) of patients in Part B (IV) experienced 1 or more TEAEs during the study. A total of 111 of 167 patients (66.5%) in Part A (IM) and 40 of 61 patients (65.6%) in Part B (IV) experienced a serious TEAE (**Table *11***).

Three of the 167 patients (1.8%) treated in Part A (IM) had a fatal event. All 3 deaths were considered unrelated to JZP-458. None of the patients in Part B (IV) died. Treatment- emergent adverse events leading to study drug discontinuation were experienced by 23 of 167 patients (13.8%) in Part A (IM) and 20 of 61 patients (32.8%) in Part B.

Table 11. Summary of treatment-emergent adverse events (Safety Analysis Set)

Number (%) of Participants with:	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Any TEAEs	32 (97.0)	83 (100)	49 (96.1)	164 (98.2)	60 (98.4)
Serious TEAEs	20 (60.6)	57 (68.7)	34 (66.7)	111 (66.5)	40 (65.6)
Treatment-related TEAEs	21 (63.6)	66 (79.5)	39 (76.5)	126 (75.4)	55 (90.2)
Treatment-related serious TEAEs	5 (15.2)	28 (33.7)	15 (29.4)	48 (28.7)	29 (47.5)
Grade 3 or 4 TEAEs	23 (69.7)	73 (88.0)	46 (90.2)	142 (85.0)	52 (85.2)
Treatment-related Grade 3 or 4 TEAEs	14 (42.4)	46 (55.4)	28 (54.9)	88 (52.7)	38 (62.3)
TEAEs leading to study drug discontinuation	3 (9.1)	14 (16.9)	6 (11.8)	23 (13.8)	20 (32.8)
Treatment-related TEAEs leading to study drug discontinuation	2 (6.1)	14 (16.9)	6 (11.8)	22 (13.2)	20 (32.8)
TEAEs leading to death	1 (3.0)	2 (2.4)	0	3 (1.8)	0
Treatment-related TEAEs leading to death	0	0	0	0	0

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; F = Friday; IM = intramuscular; IV = intravenous; MedDRA = Medical Dictionary for Regulatory Activities; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.
Percentages were calculated with the number of participants in the Safety Analysis Set as the denominator.
Adverse events were coded to SOC and PT using MedDRA 22.1. The severity of AEs was recorded using CTCAE 5.0.
A TEAE was defined as any event with an onset date on or after the first dose of study treatment through the end of the study or any ongoing event that worsened in severity after the date of the first dose of study treatment through the end of the study.

Treatment-Emergent Adverse Events

Treatment-emergent adverse events occurring in at least 10% of patients in the IM total or IV group are summarised, by descending frequency of occurrence in the overall IM group, in **Table *12***.

Overall in Part A (IM), the most frequent TEAEs were anaemia (54.5% of patients [91/167]), platelet count decreased (44.9% [75/167]), neutrophil count decreased (43.7% [73/167]), and vomiting (42.5% [71/167]).

Overall in Part B (IV), the most frequent TEAEs were vomiting (65.6% [40/61]), nausea (47.5% [29/61]), and anaemia (37.7% [23/61]).

Table 12. Summary of treatment-emergent adverse events occurring in ⩾ 10% of patients in the intramuscular total or intravenous group and by preferred term (Safety)

Preferred Term, n (%)	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Number of participants with at least 1 TEAE	32 (97.0)	83 (100)	49 (96.1)	164 (98.2)	60 (98.4)
Anaemia	13 (39.4)	49 (59.0)	29 (56.9)	91 (54.5)	23 (37.7)
Platelet count decreased	13 (39.4)	37 (44.6)	25 (49.0)	75 (44.9)	14 (23.0)
Neutrophil count decreased	14 (42.4)	34 (41.0)	25 (49.0)	73 (43.7)	11 (18.0)
Vomiting	12 (36.4)	42 (50.6)	17 (33.3)	71 (42.5)	40 (65.6)
Febrile neutropenia	10 (30.3)	28 (33.7)	21 (41.2)	59 (35.3)	14 (23.0)
Pyrexia	10 (30.3)	36 (43.4)	13 (25.5)	59 (35.3)	13 (21.3)
Nausea	9 (27.3)	31 (37.3)	18 (35.3)	58 (34.7)	29 (47.5)
Fatigue	10 (30.3)	32 (38.6)	13 (25.5)	55 (32.9)	15 (24.6)

Preferred Term, n (%)	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Decreased appetite	7 (21.2)	28 (33.7)	16 (31.4)	51 (30.5)	15 (24.6)
White blood cell count decreased	12 (36.4)	26 (31.3)	12 (23.5)	50 (29.9)	11 (18.0)
Stomatitis	8 (24.2)	23 (27.7)	17 (33.3)	48 (28.7)	18 (29.5)
Headache	12 (36.4)	23 (27.7)	12 (23.5)	47 (28.1)	11 (18.0)
Alanine aminotransferase increased	6 (18.2)	29 (34.9)	10 (19.6)	45 (26.9)	18 (29.5)
Abdominal pain	5 (15.2)	26 (31.3)	13 (25.5)	44 (26.3)	10 (16.4)
Diarrhoea	5 (15.2)	23 (27.7)	12 (23.5)	40 (24.0)	11 (18.0)
Back pain	9 (27.3)	22 (26.5)	8 (15.7)	39 (23.4)	10 (16.4)
Lymphocyte count decreased	8 (24.2)	21 (25.3)	9 (17.6)	38 (22.8)	8 (13.1)
Pain in extremity	8 (24.2)	18 (21.7)	10 (19.6)	36 (21.6)	4 (6.6)
Aspartate aminotransferase increased	5 (15.2)	24 (28.9)	6 (11.8)	35 (21.0)	13 (21.3)
Sinus tachycardia	5 (15.2)	19 (22.9)	8 (15.7)	32 (19.2)	5 (8.2)
Hyperglycaemia	7 (21.2)	14 (16.9)	7 (13.7)	28 (16.8)	11 (18.0)
Hypokalaemia	3 (9.1)	14 (16.9)	11 (21.6)	28 (16.8)	8 (13.1)
Dehydration	5 (15.2)	13 (15.7)	8 (15.7)	26 (15.6)	5 (8.2)
Constipation	4 (12.1)	13 (15.7)	8 (15.7)	25 (15.0)	12 (19.7)
Cough	5 (15.2)	11 (13.3)	9 (17.6)	25 (15.0)	7 (11.5)
Weight decreased	1 (3.0)	13 (15.7)	9 (17.6)	23 (13.8)	6 (9.8)
Contusion	4 (12.1)	8 (9.6)	8 (15.7)	20 (12.0)	3 (4.9)
Hypoalbuminaemia	4 (12.1)	12 (14.5)	3 (5.9)	19 (11.4)	6 (9.8)
Arthralgia	5 (15.2)	9 (10.8)	4 (7.8)	18 (10.8)	5 (8.2)
Blood bilirubin increased	2 (6.1)	12 (14.5)	4 (7.8)	18 (10.8)	5 (8.2)
Oropharyngeal pain	2 (6.1)	10 (12.0)	6 (11.8)	18 (10.8)	9 (14.8)
Rhinorrhoea	4 (12.1)	10 (12.0)	4 (7.8)	18 (10.8)	4 (6.6)
Hypocalcaemia	4 (12.1)	9 (10.8)	4 (7.8)	17 (10.2)	4 (6.6)
Anxiety	2 (6.1)	9 (10.8)	5 (9.8)	16 (9.6)	7 (11.5)
Insomnia	5 (15.2)	8 (9.6)	3 (5.9)	16 (9.6)	8 (13.1)
Upper respiratory tract infection	3 (9.1)	7 (8.4)	3 (5.9)	13 (7.8)	9 (14.8)

Preferred Term, n (%)	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Drug hypersensitivity	2 (6.1)	8 (9.6)	2 (3.9)	12 (7.2)	12 (19.7)

Abbreviations: F = Friday; IM = intramuscular; IV = intravenous; MedDRA = Medical Dictionary for Regulatory Activities; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; PT = preferred term; TEAE = treatment-emergent adverse event.
Percentages were calculated with the number of participants in the Safety Analysis Set as the denominator.
Adverse events were coded to PT using MedDRA 22.1.
Preferred terms were sorted by decreasing order of IM total frequency.
A TEAE was defined as any event with an onset date on or after the first dose of study treatment through the end of the study or any ongoing event that worsened in severity after the date of the first dose of study treatment through the end of the study.
Participants who reported a TEAE more than once within a PT were counted only once for that PT.

Frequency of Treatment-Emergent Adverse Events by Severity

Overall in Part A (IM), the most common (i.e., those occurring ≥ 20% patients overall) Grade 3 or 4 TEAEs were anaemia (45.5% of patients [76/167]), neutrophil count decreased (43.1% of patients [72/167]), platelet count decreased (35.9% of patients [60/167]), febrile neutropenia 35.9% of patients [60/167]), and white blood cell count decreased (25.7% of patients [43/167]).

Further Grade 3 or 4 TEAEs were reported in the System Organ Classes, gastrointestinal disorders (26.9% [45/167]), General disorders and administration site conditions (8.4% [14/167]), Immune system disorders (6.0% [10/167]), Infections and infestations (25.1% [42/167]), Injury, poisoning and procedural (4.2% [7/167]), Investigation (64.7% [108/167]), Metabolism and nutrition (22.2% [37/167]), Musculoskeletal and connective tissue disorders (7.8% [13/167]), Nervous system disorders (8.4% [14/167]), and Renal and urinary disorders (6.6% [11/167]).

In Part B (IV), the most common (i.e., those occurring ≥ 20% patients overall) Grade 3 or 4 TEAE was anaemia (32.8% of patients [20/61]), febrile neutropenia (23% of patients [14/61], platelet count decreased and ALT increased (21.3% each of patients [13/61].

Further Grade 3 or 4 TEAEs were reported in the System Organ Classes, gastrointestinal disorders (29.5% [18/61]), General disorders and administration site conditions (3.3% [2/61]), Immune system disorders (21.3% [13/61]), Infections and infestations (9.8% [6/61]), Injury, poisoning and procedural (6.6% [4/61]), Investigation (45.9% [28/61]), Metabolism and nutrition (19.7% [12/61]), Musculoskeletal and connective tissue disorders (3.3% [2/61]), Nervous system disorders (14.8% [9/61]), and Renal and urinary disorders (4.9% [3/61]).

Treatment-Related Treatment-Emergent Adverse Events

Overall in Part A (IM), the most frequent (i.e., those occurring in ≥ 10% of patients) treatment-related TEAEs were nausea (24.0% [40/167]), vomiting (23.4% [39/167]), neutrophil count decreased (22.2% [37/167]), anaemia (18.0% [30/167]), decreased appetite and ALT increased (14.4% [2/167], , platelet count decreased (13.2% [23/167]), fatigue (13.8% [23/167]), white blood cell count decreased (11.4% [19/167]), AST increased (10.8% [18/167]) and abdominal pain and AST increased (10.2% [17/167] each).

In Part A (IM), the most frequently reported (i.e., those occurring in ≥ 5% of patients overall) treatment-related Grade 3 or 4 TEAEs were neutrophil count decreased (21.6% [36/167]), anaemia (13.8% [23/167]), platelet count decreased (11.4% [19/167]), white blood cell count decreased (9.6% [16/167]), febrile neutropenia (10.2% [17/167]), ALT increased (7.8% [13/167]), lymphocyte count decreased (6.6% [11/167]), and nausea (5.4% [9/167]).

Overall in Part B (IV), the most frequent (i.e., those occurring in ≥ 10% of patients) treatment-related TEAEs were vomiting (59.0% [36/61]), nausea (44.3% [27/61]), ALT increased (18.0% [8/61]), drug hypersensitivity (16.4% [10/61]) and anaemia and hyperglycaemia (11.5% [7/61] each).

In Part B (IV), the most frequently reported (i.e., those occurring in ≥ 5% of patients overall) treatment-related Grade 3 or 4 TEAEs were vomiting (13.1% [8/61]), drug hypersensitivity, nausea and ALT increased (11.5% [7/61] each), anaemia and hyperglycaemia (9.8% [6/61] each) and neutrophil count decrease, platelet count decreased, and WBC count decreased (6.6% of patients [4/61] each).

2.5.8.3. Serious adverse event/deaths/other significant events

Serious Treatment-Emergent Adverse Events

Overall, in Part A (IM), 66.5% of patients (111/167) experienced at least 1 serious TEAE during the study (Table 19). The most frequently (i.e., those occurring in at least 5% of patients) reported serious TEAEs included febrile neutropenia (31.7% [53/167]), pyrexia (11.4% [19/167]), dehydration (9.0% [15/167]), sepsis (7.8% [13/167]), stomatitis and vomiting (6.6% [11/167] each) and nausea (5.4% [9/167]).

The majority of the reported serious TEAEs in Part A (IM) were considered not related to RC-P; 28.7% of patients (48/167) had serious TEAEs related to RC-P. Treatment-related serious TEAEs reported by 2 or more patients included: febrile neutropenia (8.4% [14/167]); drug hypersensitivity (4.8% [8/167]); nausea and pancreatitis (3.6% [6/167] each); pancreatitis acute (3.0% [5/167]); vomiting and anaphylactic reaction (1.8% [3/167] each); colitis, sepsis, hyperammonaemia, hyperglycaemia, and pulmonary embolism (1.2% [2/167] each).

Overall, in Part B (IV), 65.6% of participants (40/61) experienced at least 1 serious TEAE during the study. The most frequently (i.e., those occurring in at least 5% of participants) reported serious TEAEs that included febrile neutropenia (23.0% of participants [14/61]), vomiting (9.8% of participants [6/61]) and vomiting and, drug hypersensitivity and hyperglycaemia (8.2% of participants [5/61] each), and nausea (6.6% of participants [4/61] each).

The majority of the reported serious TEAEs in Part B (IV) were considered related to RC-P treatment. Overall, 47.5% (29/61) had serious TEAEs that were considered related to RC-P. Treatment related serious TEAEs reported by 2 or more patients included vomiting (9.8% [6/61]), drug hypersensitivity and hyperglycaemia (8.2% of patients [5/61] each), nausea (6.6% [4/61] each), febrile neutropenia and infusion related reactions (4.9% [3/61] each), and ALT increased hypoglycaemia and pancreatitis (3.3% [2/61] each).

Table 13. Summary of serious treatment-emergent adverse events occurring in ⩾ 2% of patients in the intramuscular total or intravenous group and by preferred term (Safety Analysis Set)

System Organ Class Preferred Term, n (%)	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Number of participants with at least 1 serious TEAE	20 (60.6)	57 (68.7)	34 (66.7)	111 (66.5)	40 (65.6)
Blood and lymphatic system disorders	10 (30.3)	26 (31.3)	20 (39.2)	56 (33.5)	15 (24.6)
Febrile neutropenia	9 (27.3)	24 (28.9)	20 (39.2)	53 (31.7)	14 (23.0)
Anaemia	0	3 (3.6)	1 (2.0)	4 (2.4)	2 (3.3)
Gastrointestinal disorders	6 (18.2)	17 (20.5)	12 (23.5)	35 (21.0)	12 (19.7)
Stomatitis	3 (9.1)	4 (4.8)	4 (7.8)	11 (6.6)	2 (3.3)
Vomiting	1 (3.0)	8 (9.6)	2 (3.9)	11 (6.6)	6 (9.8)
Nausea	2 (6.1)	6 (7.2)	1 (2.0)	9 (5.4)	4 (6.6)
Pancreatitis	0	3 (3.6)	3 (5.9)	6 (3.6)	2 (3.3)
Pancreatitis acute	0	4 (4.8)	1 (2.0)	5 (3.0)	0
General disorders and administration site conditions	3 (9.1)	12 (14.5)	8 (15.7)	23 (13.8)	3 (4.9)
Pyrexia	3 (9.1)	8 (9.6)	8 (15.7)	19 (11.4)	3 (4.9)
Immune system disorders	2 (6.1)	8 (9.6)	2 (3.9)	12 (7.2)	7 (11.5)
Drug hypersensitivity	2 (6.1)	4 (4.8)	2 (3.9)	8 (4.8)	5 (8.2)
Infections and infestations	5 (15.2)	16 (19.3)	16 (31.4)	37 (22.2)	5 (8.2)
Sepsis	2 (6.1)	5 (6.0)	6 (11.8)	13 (7.8)	3 (4.9)
Enterocolitis infectious	0	2 (2.4)	3 (5.9)	5 (3.0)	0
Corona virus infection	2 (6.1)	0	2 (3.9)	4 (2.4)	1 (1.6)
Injury, poisoning, and procedural complications	1 (3.0)	3 (3.6)	5 (9.8)	9 (5.4)	5 (8.2)
Toxicity to various agents	1 (3.0)	1 (1.2)	3 (5.9)	5 (3.0)	1 (1.6)
Infusion-related reaction	0	0	0	0	3 (4.9)
Investigations	0	3 (3.6)	7 (13.7)	10 (6.0)	8 (13.1)
Platelet count decreased	0	1 (1.2)	1 (2.0)	2 (1.2)	2 (3.3)
Alanine aminotransferase increased	0	0	0	0	2 (3.3)

System Organ Class Preferred Term, n (%)	IM 25 mg/m² MWF (N = 33)	IM 37.5 mg/m² MWF (N = 83)	IM 25 (MW)/ 50 (F) mg/m² (N = 51)	IM Total (N = 167)	IV 25 (MW)/ 50 (F) mg/m² (N = 61)
Metabolism and nutrition disorders	4 (12.1)	10 (12.0)	7 (13.7)	21 (12.6)	7 (11.5)
Dehydration	3 (9.1)	7 (8.4)	5 (9.8)	15 (9.0)	1 (1.6)
Hyperglycaemia	0	1 (1.2)	1 (2.0)	2 (1.2)	5 (8.2)
Hypoglycaemia	1 (3.0)	0	0	1 (0.6)	2 (3.3)
Nervous system disorders	3 (9.1)	5 (6.0)	6 (11.8)	14 (8.4)	8 (13.1)
Seizure	0	2 (2.4)	0	2 (1.2)	2 (3.3)
Renal and urinary disorders	1 (3.0)	4 (4.8)	4 (7.8)	9 (5.4)	2 (3.3)
Acute kidney injury	1 (3.0)	4 (4.8)	3 (5.9)	8 (4.8)	1 (1.6)

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; F = Friday; IM = intramuscular; IV = intravenous; MedDRA = Medical Dictionary for Regulatory Activities; MW = Monday, Wednesday; MWF = Monday, Wednesday, Friday; PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.
Percentages were calculated with the number of participants in the Safety Analysis Set as the denominator.
Adverse events were coded to SOC and PT using MedDRA 22.1. The severity of TEAEs was recorded using CTCAE v5.0.
SOC and PT were sorted alphabetically and in decreasing order of total IM frequency, respectively.
A TEAE was defined as any event with an onset date on or after the first dose of study treatment through the end of the study or any ongoing event that worsened in severity after the date of the first dose of study treatment through the end of the study.
Participants who reported a TEAE more than once within a SOC/PT were counted only once for that SOC/PT.

Deaths

Three patients (1 in the 25 mg/m2 MWF dose cohort and 2 in the 37.5 mg/m2 MWF dose cohort) died. Grade 5 (fatal) serious TEAEs included sepsis, aspiration pneumonia, and multiple organ dysfunction syndrome; none of these fatal TEAEs were related to RC-P.

Treatment emerged adverse events of interest

Adverse events of interest for asparaginase include allergic reactions, pancreatitis, and thrombosis (Kearney, 2009; Pieters, 2011; Stock, 2011; Plourde, 2014; Hijya, 2016; Asparlas, 2018; Kloos, 2020). The analysis of TEAEs of special interest (allergic reactions and pancreatitis and thrombosis) were pre-planned.

Allergic Reactions (Including Hypersensitivity and Anaphylaxis)

In Part A (IM), allergic reactions regardless of causality, inclusive of hypersensitivity and anaphylaxis, have occurred in 66 of 167 patients (39.5%). However, only 18 patients (10.8%) had at least 1 event that was considered related to RC-P. The most frequently (i.e., those occurring in at least 5% of patients) reported events related to allergic reactions included rash maculopapular (7.8% [13/167]); drug hypersensitivity (7.2% [12/167]), allergic transfusion reaction (6.0% [10/167]) and drug eruption and rash (5.4% [13/167] each). Study drug-related allergic reactions included drug hypersensitivity (6.0% [10/167]); anaphylactic reaction and rash maculopapular (1.8% [3/167] each), rash erythematous (1.2% [2/167]); and rash, and urticaria (0.6% [1/167] each).

A total of 10 patients experienced Grade 3 study-drug related allergic reactions which included drug hypersensitivity (7 patients) and anaphylactic reaction (3 patients). Of the 18 patients who had allergic reaction, inclusive of hypersensitivity and anaphylaxis, that were related to study drug, 10 patients discontinued RC-P. .

In Part B (IV), allergic reactions regardless of causality, inclusive of hypersensitivity and anaphylaxis, have occurred in 32 of 61 patients (52.4%), of which 16 patients (26.2.%) had at least 1 event that was considered related to RC-P. The most frequently (i.e., those occurring in at least 5% of patients) reported events related to allergic reactions included drug hypersensitivity (19.7% [12/61]) Rash maculopapular (9.8% [6/61]), and catheter site rash and infusion-related reaction (6.6% [4/61] each). Study drug-related allergic reactions included drug hypersensitivity in 10 patients and anaphylactic reaction, hypersensitivity, and urticaria in 1 patient each; all of these reactions resolved.

Of the 16 patients who had allergic reactions, inclusive of hypersensitivity and anaphylaxis, that were related to study drug, 13 patients discontinued RC-P.

Pancreatitis

Twelve of 167 patients (7.2%) in Part A (IM) experienced an event of pancreatitis (PT = pancreatitis acute [5patients] and pancreatitis [8 patients]; including 1 patient who experienced AEs of both preferred term [PT]), all of which were considered related to RC-P. Of these patients, 10 patients experienced an event of ≥ Grade 3 pancreatitis that was considered related to RC-P and lead to study drug
discontinuation.

Three of 61 patients (4.9%) in Part B (IV) experienced an event of pancreatitis. Of these 2 patients experienced an event of ≥ Grade 3 pancreatitis that was considered related to RC-P and led to study drug discontinuation.

Thrombosis

Three of 167 patients (1.8%) in Part A (IM) experienced a TEAE of thrombosis (PTs of superior sagittal sinus thrombosis [1 patient], pulmonary embolism [2 patients], and jugular vein thrombosis [2 patients]), of these 2 patients (1.2%) had at least 1 event that was considered related to study drug (superior sagittal sinus thrombosis [1 patient], pulmonary embolism [2 patients], and jugular vein thrombosis [1 patient]). None of the events of thrombosis led to RC-P discontinuation.

Two of 61 patients (3.3%) in Part B (IV) experienced a TEAE of thrombosis (PTs of pulmonary embolism and deep vein thrombosis [1 patient each]_, of these, 1 patient (1.6%) had at least 1 event that was considered related to RC-P (deep vein thrombosis). The TEAE of deep vein thrombosis led to study drug discontinuation.

Hepatotoxicity

Hepatic toxicity (inclusive of narrow SMQ of liver-related investigations, signs, and symptoms) was observed in 57 patients (34.1%) in Part A (IM), and 34 patients (20.4%) experienced events assessed as related to RC-P. The most frequently (i.e., those occurring in at least 5% of patients) reported hepatic toxicity inclusive of standardised MedDRA queries (SMQ) of liver-related investigations related to RC-P, included ALT increased (14.4% of patients [24/167]), AST increased (10.8% of patients [18/167]), and blood bilirubin increased (6.6% of patients [11/167]).

Hepatic toxicity (inclusive of narrow SMQ of liver-related investigations, signs, and symptoms) was observed in 20 patients (32.8%) in Part B (IV), with 13 patients (21.3%) experienced events assessed as related to RC-P. The most frequently (i.e., those occurring in at least 5% of patients) reported hepatic toxicity inclusive of SMQ of liver-related investigations related to RC-P, included ALT increased (18.0% of patients [11/61]) and AST increased (9.8% [6/61]).

Hyperglycaemia

Hyperglycaemia was observed in 28 of 167 patients (16.8%) in Part A (IM), with 15 of 167 patients (9.0%) of the reported events were assessed as related to RC-P.

Hyperglycaemia was observed in 11 of 61 patients (18.0%) in Part B (IV), and 7 of 61 patients (11.5%) of the reported were events assessed as related to RC-P.

2.5.8.4. Laboratory findings

Given the study population and that these patients received multi-agent chemotherapy, abnormal chemistry parameters and haematology parameters were expected during the course of the study. As such, shifts in chemistry parameters were observed from baseline in albumin, ALT, AST, bilirubin, glucose, triglycerides, and cholesterol levels, and shifts in haematology parameters were observed, particularly in neutrophil count, haemoglobin and platelet count. Shifts in these parameters are consistent in patients with ALL/LBL receiving multi-agent chemotherapeutic regimens, including asparaginase.

Shifts in coagulation parameters showed no discernible clinically meaningful differences from baseline.

2.5.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.5.8.6. Safety in special populations

No special populations were evaluated in the study.

Review of the final data by four age groups (<6 years, ≥ 6 to 12 years, ≥ 12 to 18 years and ≥ 18 years) showed no apparent increase in the rate of treatment-emergent adverse events (TEAEs) in any age subgroups across dosing Cohort (**Table *14***).

The most common TEAEs across dosing Cohort and age subgroup were abnormal laboratory parameters (hematology and chemistry), vomiting, pyrexia, nausea, and febrile neutropenia. These were expected TEAEs which are usually reported and observed in patients with acute lymphoblastic leukaemia and lymphoblastic lymphoma (ALL/LBL) receiving multi agent chemotherapy, including asparaginases.

Certain AEs appeared to be more frequent in a certain age group. For example, back pain seemed to be more frequently reported in the older age group (≥ 18 years group) across all dosing cohorts, although this trend was not apparent for most of the frequently observed TEAEs. There were more case of pancreatitis and thrombosis in children aged 12 years and older than in children of the age 6-12 years. For hepatic AEs, no trend in the rate of events by age subgroup was seen. Due to the small number of participants in each age subgroup and the small number of events, the significance of this observation is unclear.

Table 14. Summary of treatment-emergent adverse events by age subgroup

Number (%) of participants with		< 6 Years	≥ 6 to < 12 Years	≥ 12 to < 18 Years	≥ 18 Years
Number of Participants in each dosing Cohort, n	IM 25 mg/m^2	9	9	7	8
	IM 37.5 mg/m^2	24	34	20	5
	IM 25/25/50 mg/m^2	11	14	18	8
	IV 25/25/50 mg/m^2	20	14	17	10
Any TEAEs	IM 25 mg/m^2	8 (88.9)	9 (100)	7 (100)	8 (100)
	IM 37.5 mg/m^2	24 (100)	34 (100)	20 (100)	5 (100)
	IM 25/25/50 mg/m^2	11 (100)	12 (85.7)	18 (100)	8 (100)
	IV 25/25/50 mg/m^2	20 (100)	13 (92.9)	17 (100)	10 (100)
Treatment-related TEAEs	IM 25 mg/m^2	7 (77.8)	4 (44.4)	5 (71.4)	5 (62.5)
	IM 37.5 mg/m^2	17 (70.8)	26 (76.5)	18 (90.0)	5 (100)
	IM 25/25/50 mg/m^2	7 (63.6)	8 (57.1)	17 (94.4)	7 (87.5)
	IV 25/25/50 mg/m^2	19 (95.0)	13 (92.9)	14 (82.4)	9 (90.0)
Serious TEAEs	IM 25 mg/m^2	6 (66.7)	5 (55.6)	5 (71.4)	4 (50.0)
	IM 37.5 mg/m^2	14 (58.3)	25 (73.5)	14 (70.0)	4 (80.0)
	IM 25/25/50 mg/m^2	5 (45.5)	10 (71.4)	14 (77.8)	5 (62.5)
	IV 25/25/50 mg/m^2	13 (65.0)	8 (57.1)	13 (76.5)	6 (60.0)
Treatment-related serious TEAEs	IM 25 mg/m^2	4 (44.4)	0	1 (14.3)	0
	IM 37.5 mg/m^2	6 (25.0)	13 (38.2)	7 (35.0)	2 (40.0)
	IM 25/25/50 mg/m^2	2 (18.2)	4 (28.6)	7 (38.9)	2 (25.0)
	IV 25/25/50 mg/m^2	9 (45.0)	5 (35.7)	10 (58.8)	5 (50.0)
Grade 3 or 4 TEAEs	IM 25 mg/m^2	8 (88.9)	6 (66.7)	4 (57.1)	5 (62.5)
	IM 37.5 mg/m^2	20 (83.3)	31 (91.2)	17 (85.0)	5 (100)
	IM 25/25/50 mg/m^2	10 (90.9)	10 (71.4)	18 (100)	8 (100)
	IV 25/25/50 mg/m^2	16 (80.0)	12 (85.7)	15 (88.2)	9 (90.0)
Treatment-related Grade 3 or 4 TEAEs	IM 25 mg/m^2	7 (77.8)	2 (22.2)	3 (42.9)	2 (25.0)
	IM 37.5 mg/m^2	10 (41.7)	21 (61.8)	12 (60.0)	3 (60.0)
	IM 25/25/50 mg/m^2	3 (27.3)	7 (50.0)	13 (72.2)	5 (62.5)
	IV 25/25/50 mg/m^2	14 (70.0)	9 (64.3)	10 (58.8)	5 (50.0)

Abbreviations: IM = intramuscular; IV = intravenous; TEAE = treatment-emergent adverse event.

2.5.8.7. Immunological events

In Part A (IM), a total of 216, 513, and 322 samples were collected from patients in Cohort 1a (IM 25 mg/m^2 MWF), Cohort 1b (37.5 mg/m^2 MWF), and Cohort 1c (25 [MW]/50 [F] mg/m^2, respectively. These samples were collected from Course 1 through the end of study, with the majority of samples collected form Course 1 through Course 6. Anti-RC-P antibody data were available from a median (range) of 5.0 (1 to 14) courses from 33 patients in Cohort 1a, 4.0 (1 to 15) courses from 83 patients in Cohort 1b, and 4.0 (1 to 11) courses from 51 patients in Cohort 1c.

In Part B (IV), a total of 344 samples were collected from patients after IV administration of 25 (MW)/50 (F) mg/m^2. Anti-RC-P antibody data were available from a median (range) of 3.0 (1 to 15) courses from 61 patients in Part B (IV).

Eighty-two of 167 patients (49.1%) who received IM RC-P had confirmed positive ADA toward RC-P. Of these 82 ADA positive patients, 11 experienced a treatment-related hypersensitivity reaction during the study, and 4 developed NAb. A total of 85 of 167 (50.9%) patients were ADA negative toward RC-P, and 7 of these 85 patients experienced treatment-related hypersensitivity reaction during the study.

A posthoc analysis was conducted using only participants with at least 1 postdose evaluable ADA value. A total of 77 of 165 participants (46.7%) who received IM RC-P had confirmed positive ADA toward RC-P. Of these 77 ADA positive participants, 11 experienced a treatment-related hypersensitivity reaction during the study, and 4 developed NAb. A total of 88 of 165 participants (53.3%) who received IM RC-P were ADA negative toward RC-P, and 6 experienced a treatment-related hypersensitivity reaction during the study.

Seven patients were ADA-positive patients predose 1, all became ADA negative at least once during the study. Predose ADA positive status did not affect Course 1 SAA levels; all 7 predose ADA positive patients had NSAA levels ≥ 0.1 IU/mL at all available 48- and 72-hour timepoints during Course 1.

In part B (IV) 34 of 61 (55.7%) patients had confirmed positive ADA toward RC-P. Of these 34 ADA positive patients, 12 patients experienced a treatment-related hypersensitivity reaction during the study and 2 developed NAb. A total of 27 of 61 patients (44.3%) who received IV RC-P were ADA negative toward RC-P and 4 of these patients experienced a treatment-related hypersensitivity reaction during the study. None of the patients had NAbs.

A posthoc analysis was conducted using only participants with at least 1 postdose evaluable ADA value. A total of 33 of 60 participants (55.0%) who received IV RC-P had confirmed positive ADA toward RC-P. Of these 33 ADA-positive participants, 11 experienced a treatment-related hypersensitivity reaction during the study, and 2 developed positive NAb. A total of 27 of 60 participants (45.0%) who received IV RC-P were ADA negative toward RC-P, and 5 experienced a treatment-related hypersensitivity reaction during the study.

2.5.8.8. Safety related to drug-drug interactions and other interactions

No interactions studies have been performed.

2.5.8.9. Discontinuation due to adverse events

Overall, in Part A (IM), 23 of 167 patients (13.8%) experienced a TEAE that led to discontinuation of study drug. Events that led to discontinuation of study drug included drug hypersensitivity (4.2% [7/167]); pancreatitis (3.6% [6/167]), and pancreatitis acute (2.4% [4/167] each); anaphylactic reaction (1.8% [3/167]); and necrotizing fasciitis, ALT increased, and hyperammonaemia (0.6% [1/167] each). All AEs leading to discontinuation were considered related to RC-P, except for necrotizing fasciitis which was deemed related to study procedures (IM injection).

Overall in Part B (IV), 20 of 61 patients (32.8%) experienced a TEAE that led to discontinuation of RC-P. Events that led to discontinuation of RC-P included drug hypersensitivity (14.8% [9/61]), pancreatitis, vomiting and infusion-related reaction (3.3% [2/61] each) and nausea, anaphylactic reaction, hypersensitivity, hyperammonaemia encephalopathy, and deep vein thrombosis (1.6% [1/61] each). All events leading to RC-P discontinuation were considered related to study treatment.

2.5.8.10. Post marketing experience

RC-P was approved by the FDA on 30 June 2021 at a dosage of 25 mg/m^2 administered IM every 48 hours.

Through 31 January 2022, a total of 11 spontaneous cases (presenting 21 events) were reported for RYLAZE (brand name in the US). The most commonly reported events are rash (2) and drug hypersensitivity (2). No new risks have been identified based on post-marketing sources through 31 January 2022.

2.5.9. Discussion on clinical safety

Safety data for RC-P is based on study JZP458-201, a single arm ongoing Phase 2/3 study in paediatric and adult patients with ALL or LBL, who developed an allergic reaction or silent inactivation to E. coli-derived asparaginase. The safety data base includes a total of 167 patients who were treated with different treatment regimens of IM RC-P and 61 patients treated with IV RC-P.

Most patients in the Safety data Analysis set were below the age of 18 years.

Adverse events (AEs)

For both part A (IM) and part B (IV) the most common Grade 3 or 4 TAES were haematological AEs including anaemia, neutrophil count decrease, platelet count decreased, febrile neutropenia and white blood cell count decreased. Further, Grade 3 Gastrointestinal disorders, Infections and infestations and Metabolism and nutrition disorders, were commonly reported.

Given the single arm study design and the extensive co-medication (including chemotherapy) that the ALL patients receive, interpretation of the safety data is hampered. Even so, while numeric differences are seen, the general safety profile of RC-P seems to be reasonable comparable to crisantaspase.

Only limited numbers of patients were included per dosing cohort and the study was not designed or powered to show differences in safety between doses studied or between the IM versus the IV routes of administration. Comparison of safety data between different cohorts should therefore be done with caution. Nevertheless, provided data does not indicate a consistent difference in the percentage of patients with AEs between dose cohorts in Part A. More treatment related serious AEs and AEs leading to study drug discontinuation were reported for patients treated in part B than for patients treated in part A of the study (47.5% vs 28.7% and 32.8% vs 13.8%, respectively). For part B (IV) more of the reported SAE's were considered study drug related, the incidence of all reported SAEs was comparable for patients who received IM and IV RC-P (65.6% vs 66.5%, respectively).

Serious adverse events (SAEs) and deaths

The majority of the reported serious TEAEs in Part A (IM) were considered not related to RC-P; 28.7% of patients (48/167) had serious TEAEs that were considered related to RC-P. Treatment-related serious TEAEs reported by 2 or more patients included: febrile neutropenia (8.4% [14/167]); drug hypersensitivity (4.8% [8/167]); nausea and pancreatitis (3.6% [6/167] each); pancreatitis acute (3.0% [5/167]); vomiting and anaphylactic reaction (1.8% [3/167] each); colitis, sepsis, hyperammonaemia, hyperglycaemia, and pulmonary embolism (1.2% [2/167] each).

The majority of the reported serious TEAEs in Part B (IV) were considered related to RC-P. Overall, 47.5% of patients (29/61) had serious TEAEs that were considered related to RC-P. Treatment related serious TEAEs reported by 2 or more patients included vomiting (9.8% [6/61]), drug hypersensitivity and hyperglycaemia (8.2% [54/61 each]), nausea (6.6% [4/61]), febrile neutropenia and infusion-related reaction (4.9% [3/61] each) and ALT increased, hypoglycaemia and pancreatitis and hyperglycaemia (3.3% [2/61] each).

Three patients (1 in the 25 mg/m^2 MWF dose cohort and 2 in the 37.5 mg/m^2 MWF dose cohort) died. Grade 5 (fatal) serious TEAEs included sepsis, aspiration pneumonia, and multiple organ dysfunction syndrome; none of these fatal TEAEs were considered related to RC-P.

For crisantaspase neurotoxicity is reported including CNS depression, convulsion, encephalopathy and Posterior Reversible Encephalopathy Syndrome (PrES), is reported as risk and included in section 4.4 and 4.8 of the SmPC For RC-P a warning regarding these potential AEs is included in section 4.4 of the SmPC.

The incidence and kind of serious TAEs reported for RC-P seem to be in line with what has been reported previously for other asparaginase products.

Events that led most often to discontinuation of study drug included, hypersensitivity, pancreatitis and pancreatitis acute, and anaphylactic reactions, which are known AEs for asparaginase therapy resulting in discontinuation of asparaginase treatment.

Other Events of Special Interest (OESIs)

Allergic Reactions (Including Hypersensitivity and Anaphylaxis)

In Part A (IM), a total of 10 patients experienced Grade 3 study-drug related allergic reactions which included drug hypersensitivity (7 patients) and anaphylactic reaction (3 patients). Of the 18 patients who had allergic reaction, and that were considered related to study drug, 10 patients discontinued RC-P. In Part B (IV), of the 16 patients who had allergic reactions considered related to study drug, 13 patients discontinued RC-P.

Hypersensitivity reaction is one of the most relevant toxicities of asparaginase treatment. The reported frequency of hypersensitivity to any type of asparaginase varies considerably in published trials the reported incidence is between 10% to 30% for patients receiving asparaginase for the first time. The patients included in the current study had already experienced a hypersensitivity reaction or had silent inactivation to E. coli derived asparaginase. Specifically, more than 70% of the patients included had had a hypersensitivity reaction against *E. coli* derived asparaginase. For crisantaspase which is also indicated for patients who experienced an hypersensitivity reaction on, or had silent inactivation of *E. Coli* derived asparaginase treatment, allergic reactions are reported for 3-37% ALL patients (Hijiva, 2016). The incidence of hypersensitivity reactions in the current study are comparable to the incidences reported for crisantaspase.

Based on a review performed by the EMSO of 2 studies with pre-medications and PEG asparaginase (Burke et al., 2020), the use of pre-medication to decrease the incidence of hypersensitivity reactions with asparaginase treatment is recommended in ESMO and NCCN guidelines. Pre-medication was associated with both a lower rate of substitution of PEG asparaginase to crisantaspase asparaginase and lower acute adverse events.

The protocol of study JZP458-201 did not specify or require the use of pre-medications prior to the administration of RC-P. Upon further analysis the applicant indicated that the majority of included patients (~71% in IM total group and 59% in IV arm) did not receive pre-medications as primary prophylaxis to prevent allergic reactions and current data are inconclusive as to whether pre-medication should be recommended for all patients. A higher rate of allergic reactions and study drug discontinuations due to allergic reactions has however been noted in patients who received IV treatment compared to IM RC-P. As such, a consideration for premedication with IV use as currently proposed in SmPC section 4.2 is endorsed.

Pancreatitis

Twelve of 167 patients (7.2%) in Part A (IM) experienced an event of pancreatitis (PT = pancreatitis acute [5 patients] and pancreatitis [8 patients]; including 1 patient who experienced AEs of both preferred term [PT]), all of which were considered related to RC-P. Of these patients, 10 patients experienced an event of ≥ Grade 3 pancreatitis that was considered related to RC-P and lead to study discontinuation.

Three of the 61 patients (4.9%) in Part B (IV) experienced an event of pancreatitis. Of these 2 patients experienced and event of ≥ Grade 3 pancreatitis that was considered related to RC-P and led to study drug discontinuation

In the literature the reported incidence of asparaginase-associated pancreatitis ranges from 2% to 18% (Burke et al., 2020), in line with the observed incidence of pancreatitis for RC-P.

Thrombosis

Three of 167 patients (1.8%) in Part A (IM) experienced an TEAE of thrombosis Of which 2 (1.2%) had at least 1 event that was considered related to study drug. None of the events of thrombosis led to RC-P discontinuation. In part B (IV) 2 of 61 patients (3.3%) experienced a TEAE of thrombosis. One of those patients had at least 1 event that was considered related to RC-P (deep vein thrombosis) and led to study drug discontinuation.

In published studies, the reported incidences of thrombosis in patients receiving asparaginase-containing chemotherapy for ALL were 2% to 10%(Caruso, 2006; Raetz and Salzer, 2010), in line with the observed rates in the RC-P studies.

The use and possible type of prophylactic medications and strategies to prevent thrombosis prior to asparaginase administration was not fixed in the study protocol, use was left to investigator's discretion. A review of concomitant medications revealed that a total of 7 patients received anticoagulants, prophylactically to prevent thrombosis. In addition, 2 patients received heparin potentially for central line care or another reason. Based on these small numbers, there is insufficient evidence to recommend the use of anticoagulation for prophylaxis in prevention of thrombosis.

Hepatotoxicity

Hepatotoxicity is a commonly reported AE for asparaginase therapy, risk is increased with age, higher doses and obesity (Burke et al., 2020). For RC-P treatment hepatic toxicity was observed in 57 patients (34.1%) in Part A (IM), with 34 patients (20.4%) experienced events assessed as related to RC-P.

As of the data cut-off date, hepatic toxicity was observed in 20 patients (32.8%) in Part B (IV), with 13 patients (21.3%) experienced events assessed as related to RC-P.

The incidence seen for RC-P treatment is in line with which has been reported for other asparaginase products. For crisantaspase AEs of hepatotoxicity including hepatic steatosis, hepatic failure, cholestatic jaundice and hepatomegaly, have been described. These AEs are not reported for RC-P. However, this might be due to the limited safety data base. The applicant will monitor severe hepatotoxicity via routine pharmacovigilance activities and the information will be included in the periodic safety update reports.

Hyperglycaemia

The reported incidences of hyperglycaemia (116.8% for par A (IM) and 18.0% for part B (IV) are in line with what has been reported for other asparaginase products. Severe hyperglycaemia was reported for 2 (1.2%) of the patients treated in part A (IM) and for 5 (8.2%) of the patients treated in part B (IV), none resulted in discontinuation of study drug.

Immunogenicity

Eighty-two of 167 patients (49.1%) who received IM RC-P had confirmed positive ADA and of those, 11 experienced a treatment-related hypersensitivity reaction during the study, and 4 developed positive NAb. 7 of the 85 ADA negative patients experienced treatment-related hypersensitivity reaction during the study. Seven patients were ADA positive patients predose 1, all became ADA negative at least once during the study. Predose ADA positive status did not affect Course 1 SAA levels.

In part B (IV) 34 of 61 (55.7%) patients had confirmed positive ADA and of those, 12 experienced a treatment-related hypersensitivity reaction during the study and 2 developed positive NAb. Four of the 27 ADA experienced a treatment-related hypersensitivity reaction during the study.

As could be expected the incidence of hypersensitivity reactions is higher for patients who developed ADAs during treatment. However, the incidence of hypersensitivity reactions was comparable to what has been reported for crisantaspase in the literature.

In the study of Wang et al. (2020), none of the patients who received only *E.coli* asparaginase, cross-reacted with the Erwinia antigen. Cross-reactivity of Enrylaze with other asparaginases has not been studied. Previous studies show that there is greater cross-reactivity of plasma between native *E.-coli* derived asparaginase and PeG-asparaginase than there is between native *E.coli*-derived asparaginase and Erwinia-derived asparaginase. Potential cross-reactivity of Enrylaze with other asparaginases is expected to be minimal. This is due to the fact that *Erwinia*-derived asparaginase and *E. coli*-derived asparaginase share only 70% amino acid homology and therefore, cross-reactivity with native *E coli*–derived asparaginase is expected to be low.

Furthermore, although there is an association between antibody levels against native *E. coli*-derived asparaginase and asparaginase activity (inverse correlation), one-third of blood samples with insufficient asparaginase activity after the administration of native *E coli*-derived asparaginase tested negative for the presence of antibodies. Apart from antibodies, other factors such as insufficient dosing or increased clearance because of degradation by proteases, might influence the treatment efficacy.

In total 89.8% (95% CI: 81.3, 98.3) of participants achieved NSAA levels ≥ 0.1 IU/mL at the last 72-hour assessment during the first course of treatment in Cohort 1c (IM) and in Part B (IV) of the, 40% (95% CI: 26.4, 53.6) of patients achieved NSAA levels ≥0.1 IU/mL at the last 72-hour assessment. Given the efficacy results it seems that most of the patients who switched due to allergic reactions on *E.Coli* derived asparaginase, did not have anti-bodies that cross-reacted to RC-P resulting in RC-P inefficiency. Moreover, the risk of silent inactivity of RC-P due to cross-reactivity seems to be limited, and a recommendation for therapeutic drug monitoring (TDM) has also been included in section 4.2.

It seems that slightly more patients have developed ADAs with RC-P treatment than which has been reported in patients treated with crisantaspase, for which the incidence of ADA positive patients ranges between 8 and 33% (Albersten et al., 2001b, Albertsen et al., 2002b and Wang et al., 2003). However, for all asparaginase products the frequency of the development of anti-asparaginase antibodies is highly variable between studies. Development of ADA's is of relevance if it results in early discontinuation of asparaginase treatment, due hypersensitivity reactions and/or development of NAbs, because this impacts efficacy of the treatment. Of the 18 patients in part A (IM) who had allergic reaction, inclusive of hypersensitivity and anaphylaxis, that were related to study drug, 10 patients discontinued RC-P. Of the 16 patients in part B (IV) who had allergic reactions, inclusive of hypersensitivity and anaphylaxis, that were related to study drug, 13 patients discontinued RC-P. Overall, most patients completed their planned asparaginase treatment.

The number of participants with neutralizing antibodies (NAb) is very small (IM Part A: n=4; IV Part B: n=2). Mean SAA levels for the last 48-hour and the last 72-hour were comparable between ADA+ and ADA- subjects throughout treatment across all dosing regimens tested in the clinical study. Although no firm conclusions can be drawn based on the small number of NAb+ subjects, available data do not suggest a difference in the impact of NAb status on SAA levels.

Safety specific populations

Most patients included in the study are below the age of 18 years, therefore the safety profile of adults is largely unknown. Toxicity data and the incidence of AEs per age group (<6 years, ≥6 to <12 years, ≥12 to <18 years and >18 years) were provided.

Due to the small number of patients included in the age subgroups and the small number of events, no definitive conclusion can be drawn with regard to similarity of RC-P toxicity for patients at different ages. However, in general the safety profile of RC-P seems comparable across age subgroups. There was no consistent trend toward higher or toxicity for one of the age subgroups.

In section 4.8 of the SmPC it is mentioned that the majority of patients were <18 years of age and that the safety profile of adults above 25 years of age is has not been studied, some adverse reactions, such as hepatotoxicity, thrombosis, and pancreatitis, have been reported more frequently in adults with acute lymphoblastic leukaemia receiving other asparaginases than the paediatric patients.

2.5.10. Conclusions on the clinical safety

The safety profile of RC-P is in line with what is known from other asparaginase products. There was no clear difference in safety between different dosing regimens. Only limited number of adult patients are included in the safety database and all these patients were below the age of 25 years. The safety of RC-P in adults will be monitored post approval. Risks associated with the use of RC-P can be adequately managed with the information and warnings included in the product information.

2.6. Risk Management Plan

2.6.1. Safety concerns

None.

2.6.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.6.3. Risk minimisation measures

None.

2.6.4. Conclusion

The CHMP considers that the risk management plan version 0.3 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

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

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive

2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.8. Product information

2.8.1. User consultation

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

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Enrylaze (crisantaspase) is included in the additional monitoring list as:

- It is a new biological product

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The proposed target indication is:

'Enrylaze is indicated as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) in adult and paediatric patients (1 month and older) who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase.'

ALL and LBL are a group of rare haematological malignancies arising from precursor B cells (B-ALL/B-LBL), T cells (T-ALL/T-LBL), or both B and T cells (mixed lineage ALL/LBL). The 2008 and 2016 WHO classifications of lymphoid malignancies define B-ALL/B-LBL and T-ALL/T-LBL as single disease entities, categorised as leukaemia if there is extensive blood and marrow involvement, lymphoma if the process is confined to a mass lesion with no or minimal blood/marrow involvement, and leukaemia/lymphoma if both are present. ALL and LBL are similar in appearance and behaviour and both require intensive treatment.

Asparaginase treatment is known to contribute to overall survival of ALL/LBL patients. Asparaginase therapy is based on the inability of leukemic cells to synthesise asparagine due to lack of asparagine synthetase activity, resulting in cytotoxicity specific for leukaemic cells that rely on exogenous asparagine for their protein metabolism and survival. It should be demonstrated that the switch to this alternative asparaginase treatment results in sustained asparagine depletion (as measured by intermediate endpoint asparaginase activity).

3.1.2. Available therapies and unmet medical need

In the EU, the majority of patients diagnosed with ALL and LBL follow a standard treatment protocol in which *E. coli*-derived L-asparaginase is an important component of first line therapy.

There are currently three preparations of asparaginase for use in patients with ALL/LBL:

- Short-acting *E. coli* derived L-asparaginase (Spectrila)
- Long-acting pegylated (PEG)-asparginase*, E. coli* derived (Oncaspar)
- Short-acting *Erwinia chrysanthemi* L-asparaginase (crisantaspase) indicated for patients who develop a hypersensitivity reaction to *E. coli*-derived asparaginases (e.g. Erwinase)

Switching patients who develop clinical hypersensitivity or silent inactivation to *E. coli*-derived asparaginase to the alternative non-*E. coli*-derived preparation (e.g. Erwinase) can maintain survival benefits when adequate asparagine depletion is achieved. However, since 2016, there has been a worldwide shortage of Erwinase due to ongoing manufacturing issues.

Patients with hypersensitivity or silent inactivation to an *E. coli*-derived asparaginase have an unmet need for a reliable immunologically distinct L-asparaginase product that will allow them to continue and complete their prescribed treatment.

3.1.3. Main clinical studies

Pivotal clinical study JZP458-201 is a single arm ongoing Phase 2/3 study in paediatric and adult participants with ALL or LBL, who developed an allergic reaction or silent inactivation to an *E. coli*-derived asparaginase.

The study consisted of two parts, Part A investigating IM RC-P and Part B investigating IV 25/25/50 RC-P and multiple sub-cohorts at different dose.

As of the 22 Nov 2022 final analysis data cut-off date, 167 patients were included in part A, and 62 in part B. The median age was 10 years and the majority of patients in both study parts had experienced an allergic reaction to prior asparaginase treatment (72-90%), and B-ALL as primary disease (>74%). The median number of completed courses was 5 in part A 3 in part B.

The primary endpoint was response rate, i.e., patients with last 72-hour nadir serum asparaginase activity (NSAA) level ≥ 0.1 IU/mL during first course IM, and for the key secondary endpoint response rate with last 48 hour NSAA level ≥ 0.1 IU/mL during first course IM. Asparaginase activity is a well-established surrogacy for asparagine depletion and clinical efficacy and thereby contributing to OS gain as part of the overall treatment paradigm for ALL and LBL.

The primary and key secondary endpoints upon IM administration were to be considered met if the lower bounds of the 95% CI of the response rates exceeded 90%. Results upon IV administration were planned as exploratory.

3.2. Favourable effects

In part A of the pivotal trial, 89.8% (95% CI: 81.3, 98.3) of the patients in Cohort 1c, with the targeted dose level of 25/25/50, achieved NSAA levels ≥ 0.1 IU/mL at the last 72 hour assessment during first course IM. At the 48-hour assessment, this was 95.9% (95% CI: 90.4, 100.0).

NSAA levels ≥0.4 IU/mL during first course IM in Cohort 1c were achieved by 46.9% (95% CI: 33.0, 60.9) of patients at the last 72-hour assessment and 65.3% (95% CI: 52.0, 78.6) of patients at the last 48-hour assessment.

In Part B of the pivotal trial JZP458-201, 40% (95% CI: 26.4, 53.6) of patients achieved NSAA levels ≥ 0.1 IU/mL at the last 72 hour assessment and 89.8% (95% CI: 82.1, 97.5) of patients achieved NSAA levels ≥ 0.1 IU/mL at the last 48-hour assessment during the first course of treatment with IV 25/25/50 administration of RC-P.

NSAA levels ≥ 0.4 IU/mL were achieved by 16.9% (95% CI: 7.4, 26.5) of patients at the last 48-hour assessment with IV administration.

3.3. Uncertainties and limitations about favourable effects

The predefined target for the primary endpoint was not met and results were not fully controlled for type I error. Despite this and even allowing for a possible overestimation of the effect of Enrylaze in the clinical trial, the response rates achieved are still considered clinically relevant. Furthermore, trough asparaginase activity measurement is required for all patients which should allow adaptation of the dosing schedule or route of administration if required.

Most of the patients studied were diagnosed with ALL. However, as in clinical practice ALL and LBL are considered similar, efficacy in LBL can be based on extrapolation from results in ALL.

Only few patients with prior silent inactivation to *E. Coli* derived asparaginase were investigated, while these patients form an important part of the target population. Cross-reactivity of Enrylaze with other asparaginases has not been studied. Previous studies show that there is greater cross-reactivity of plasma between native *E.-coli* derived asparaginase and PeG-asparaginase than there is between native *E.coli*-derived asparaginase and Erwinia-derived asparaginase. Potential cross-reactivity of Enrylaze with other asparaginases is expected to be minimal. This is due to the fact that *Erwinia*-derived asparaginase and *E. coli*-derived asparaginase share only 70% amino acid homology and therefore, cross-reactivity with native *E coli*–derived asparaginase would be low.

Although eligible, patients aged 1 month to <1 year, or >25 years were not included. Treatment with PEG asparaginase and development of hypersensitivity/silent inactivation is expected to take longer than 1 month and there is no reason to assume that Enrylaze would not work in paediatric patients <1 year. Based on similar disease characteristics and treatment in patients 1 month and older, extrapolation of data to younger children aged 1 month to 1 year is acceptable. Although response rates for children between 1 month to <1 year are more uncertain, as adjustments in BSA based dosing are described for Spectrila (<1y) and due to the absence of data in this age range in the current dossier, this is acceptable considering the recommended therapeutic drug monitoring. ALL disease characteristics and treatment are not fully similar between the studied population and (older) adults. However, there is no reason to believe that RC-P will be not effective in adults >25 years of age. There are several publications for other asparaginase products (Oncaspar and nationally registered Erwinase) that indicate efficacy of asparaginase therapy in older adults as well. These have been discussed during registration of these products and resulted in an indication that is not restricted by age. Moreover, large effects of developmental processes in elimination pathways on exposure are not expected for the older age groups As such, extrapolation to patients aged 1 month to <1 year and >25 years is endorsed.

Even though not all dose regimens are investigated, it should be considered that the dosing regimens that are not tested are very similar to the dose regimens that were tested in the clinical studies. Of note, all doses in the new regimens (25 mg/m2 IM, 25 mg/m2 IV, and 50 mg/m2 IM) and dose-intervals in the new regimens (48h or 72 h) are also present in one of the dose regimens that were tested in the clinical studies. Although no exact numbers on response rate can be calculated, from pharmacokinetic principles no (large) differences in exposure and response rate are expected for regimens not tested in clinical studies. Importantly, SAA levels can be monitored in clinical practice. The alternative dosing regimens are therefore considered acceptable.

3.4. Unfavourable effects

Overall, 164 of 167 patients (98.2%) in Part A (IM) and 60 of 61 (98.4%) of patients in Part B (IV) experienced 1 or more TEAEs during the study. A total of 111 of 167 patients (66.5%) in Part A (IM) and 40 of 61 patients (65.6%) in Part B (IV) experienced a serious TEAE. A total of 3 of 167 patients (1.8%) in Part A (IM) had a fatal event, none of these were considered related to RC-P treatment. None of the patients in Part B (IV) died. Treatment-emergent adverse events leading to study drug discontinuation were experienced by 23 of 167 patients (13.8%) in Part A (IM) and 20 of 61 patients (32.8%) in Part B. Events that led to study drug discontinuation included hypersensitivity, pancreatitis and pancreatitis acute, anaphylactic reaction, ALT increased, hyperammonaemia, vomiting, infusions related reaction, nausea, encephalopathy and deep vein thrombosis.

The most frequently (i.e., those occurring in at least 5% of participants) reported serious TEAEs included febrile neutropenia (23.0% of participants [14/61]), vomiting (9.8% of participants [6/61]) and vomiting and, drug hypersensitivity and hyperglycaemia (8.2% of participants [5/61] each), and nausea (6.6% of participants [4/61] each).

For asparaginase products, hypersensitivity reactions are common events that might lead to discontinuation of asparaginase treatment. In Part A (IM), allergic reactions regardless of causality, inclusive of hypersensitivity, anaphylactic reaction, rash maculopapular and urticaria, occurred in 66 of 167 patients (39.5%). Of the patients who experienced a hypersensitivity reaction, 18 (10.8%) had at least one event that was considered related to RC-P. In Part B (IV), allergic reactions, including hypersensitivity, rash maculopapular, catheter site rash, anaphylactic reaction, infusion-related reactions and urticaria, regardless of causality, occurred in 32of 61 patients (52.4%), of which 16 patients (26.2%) had at least 1 event that was considered related to RC-P.

Eighty-two of 167 patients (49.1%) in part A (IM) who received IM RC-P had confirmed positive ADA. Of these 82 ADA positive patients, 11 experienced a treatment-related hypersensitivity reaction during the study, and 4 developed positive NAb. In part B (IV) 34 of 61 (55.7%) patients had confirmed positive ADA toward RC-P. Of these 34 ADA positive patients, 12 patients experienced a treatment-related hypersensitivity reaction during the study and 2 developed positive NAb.

3.5. Uncertainties and limitations about unfavourable effects

Given the single arm study design and the extensive co-medication (including chemotherapy) that ALL patients receive, interpretation of the safety data is hampered. However, the reported safety profile is consistent with what is known from other asparaginase.

Seven patients were already ADA positive before RC-P treatment. All these patients became ADA negative at least once during study and ADA positive status did not affect SAA levels, which can be considered reassuring. Cross-reactivity of Enrylaze with other asparaginases has not been studied. Previous studies show that there is greater cross-reactivity of plasma between native *E.-coli* derived asparaginase and PeG-asparaginase than there is between native E.coli-derived asparaginase and Erwinia-derived asparaginase. Potential cross-reactivity of Enrylaze with other asparaginases is expected to be minimal. This is due to the fact that *Erwinia*-derived asparaginase and *E. coli*-derived asparaginase share only 70% amino acid homology and therefore, cross-reactivity with native *E coli*–derived asparaginase would be expected to be low.

Most patients included in the study are below the age of 18 years, and while there is no reason to expect significant in the safety profile of adults patients, the safety of RC-P in adults will be monitored post approval.

3.6. Effects Table

Table 15. - **Effects table for Enrylaze as a component of a multi-agent chemotherapeutic regimen for the treatment of ALL and LBL in adult and paediatric patients who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase (data cut-off:22 Nov 2022)**

Effect	Short Description	Unit	Treatment IM	Treatment IV*	Uncertainties/ Strength of evidence	References
Favourable Effects						
RR last 72-hour NSAA ≥0.1 IU/mL	Proportion of patients with the last 72-hour NSAA level ≥0.1 IU/mL during the first course of **IM** RC-P.	%	89.9% (95% CI: 81.3, 98.3)	40% (95% CI: 26.4, 53.6)	IM: Predefined target (i.e. lower bounds of the 95% CI exceeding 90%) not met. Relaxed type I error control (0.08), optimistic type of 95%-CI, and some drop-out from the enrolled set to the efficacy analysis set.	(1)
RR last 48-hour NSAA ≥0.1 IU/mL	Proportion of patients with the last 48-hour NSAA level ≥0.1 IU/mL during the first course of **IM** RC-P.	%	95.9% (95% CI: 90.4, 100)	89.8% (95% CI: 82.1, 97.5)	Predefined target is met.	(1)
Unfavourable Effects						
SAEs	Proportion of patients with SAEs	%	66.5%	65.6%	The study was not designed or powered to show differences in safety between doses studied or between the IM versus the IV routes of administration.	
Discontinuation	Proportion of patients with AEs leading to study drug discontinuation	%	13.8%	32.8%		
Immunogenicity	Proportion of patients who developed ADA's	%	49.1%	55.7%	The number of participants with neutralizing antibodies (NAb) is very small (Part A [IM]: n=4; Part B [IV]: n=2) which does not allow to draw conclusions on the potential impact of NAb on SAA	

Abbreviations: IM: intramuscular; IV: intravenous; NSAA: nadir serum asparaginase activity; RR: response rate.
Notes: (1) Study JZP458-201; IV part B of the study was exploratory

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Clinical efficacy of recombinant crisantaspase produced in *Pseudomonas fluorescens* (RC-P) is claimed based on PD endpoints related to serum asparaginase activity in single arm pivotal Phase 2/3 Study JZP458-201. Asparaginase activity is considered a well-established intermediate endpoint for asparagine depletion and as such can support a claim of efficacy.

The primary endpoint was not met at the intended dose level 25/25/50 nor at one of the other dose levels, as the lower bound of the 95% CI did not exceed the predefined target of 90%. The observed sustained response rate is still considered clinically relevant especially considering the target population of patients with ALL or LBL, who developed an allergic reaction or silent inactivation to an *E. coli*-derived asparaginase, for which no (stable supply of an) alternative asparaginase treatment option exists.

Data for the IV route of administration with a M/W/F schedule indicate lower response rates compared to IM administration. This was expected based on the lower half life and experience with crisantaspase and is acceptable considering the recommended therapeutic drug monitoring for IV administration and potential to switch to an alternative administration route when needed. Although median response rates at the last 72-hour assessment do not always reach target levels, a proportion of patients still obtained sufficient asparaginase activity. The MWF IV regimen would allow patients and treating physicians to complete the regimen during the week and avoid mandatory weekend administrations. Moreover, the IV route of administration would allow to stop infusions immediately in case of acute hypersensitivity reactions and increase patient convenience when they already have a central line. The larger variety in SAA levels between patients with IV administration and option to switch to different dosing regimens is reflected in the warning regarding TDM in SmPC section 4.4.

In general the safety profile of RC-P is in line with what is known from other asparaginase products and can be adequately managed with routine risk minimisation measures.

3.7.2. Balance of benefits and risks

The study population, despite some limitations in terms of age groups included, and small percentage of patients with LBL or who had silent inactivation to *E. coli*-derived asparaginase, represents a population with limited treatment options. The observed efficacy is considered clinically relevant in this population. The study design also hampers comprehensive assessment of the risks associated with the use of Enrylaze, however, its safety profile, including common adverse reactions, can be considered reasonably established, also taking into account knowledge from other authorised asparaginase containing products.

The benefit/risk balance is therefore considered positive.

3.8. Conclusions

The overall benefit/risk balance of Enrylaze is positive, subject to the conditions stated in section ‘Recommendations.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Enrylaze is not similar to Iclusig, Blincyto, Besponsa, Kymriah and Tecartus within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus hat the benefit-risk balance of Enrylaze is favourable in the following indication:

as a component of a multi-agent chemotherapeutic regimen for the treatment of acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL) in adult and paediatric patients (1 month and older) who developed hypersensitivity or silent inactivation to E. coli-derived asparaginase.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

These conditions fully reflect the advice received from the PRAC.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0453/2021 and the results of these studies are reflected in the

Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.