29 May 2019
EMA/CHMP/257513/2019
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal Assessment report

Xyndari

International non-proprietary name: glutamine

Procedure No. EMEA/H/C/004734/0000

Note

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

Table of contents

List of abbreviations

14C	Carbon-14
15N	Nitrogen-15
ACS	acute chest syndrome
ADME	absorption, distribution, metabolism, and excretion
ADP	adenosine diphosphate
AE	Adverse Event
AIHA	autoimmune haemolytic anemia
ALT	Amino Alanine Transferase
AMP	adenosine monophosphate
ANOVA	analysis of variance
API	Active Pharmaceutical Ingredient
AR	Assessment Report
ASM	Active Substance Manufacturer
ASMF	Active Substance Master File = Drug Master File
AST	Aspartate Amino Transferase
ATP	adenosine triphosphate
b.w.	body weight
BA	bioanalytical
BID	Two Times a Day
Bpm	beats per minute
BrdU	bromodeoxyuridine
BUN	Blood Urea Nitrogen
CAC	Central Adjudication Committee
CBC	Complete Blood Count
CEP	Certificate of Suitability of the EP
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CHO	Chinese Hamster Ovary
CI	confidence interval
Cmax	maximum concentration of drug in plasma
CMH	Cochran-Mantel-Haenszel
CMS	Concerned Member State
CoA	Certificate of Analysis
Conc	concentration
CRA	Clinical Research Associate
CRF	case report form
CRO	Contract Research Organization
CSR	clinical study report
CYP	cytochrome
DAB	German Pharmacopoeia
DART	developmental and reproductive toxicity
DHR	dihydrorhodamine 123
dL	Deciliter
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPG	2,3-diphosphoglycerate
DSMB	Data and Safety Monitoring Board
DXR	doxorubicin
eCRF	Electronic Case Report Form
EDQM	European Directorate for the Quality of Medicines
EMA	European Medicines Agency
ER	Emergency Room
eSAEF	Electronic Serious Adverse Event Form
EU	European Union
EW	Early Withdrawal

FDA	Food and Drug Administration
FPM	Finished Product Manufacturer
FTIR	Fourier Transform Infrared Spectroscopy
g	Grams
G6PD	glucose-6-phosphate dehydrogenase
GC	Gaschromatography
GCP	Good Clinical Practices
GGT	gamma glutamyl transferase
GGTP	Gamma Glutamyl Transpeptide
Gln	glutamine
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
H0	null hypothesis
Hb	Hemoglobin
HbS	sickle cell Hemoglobin
HC	Hydroxycarbamide
Hct	Hematocrit
HED	human equivalent dose
HEENT	Head, Ears, Eyes, Nose and Throat
HEP	hepatocytes
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HR	high reticulocytes
HSA	human serum albumin
HU	Hydroxyurea
HUVEC	human umbilical vein endothelial cell
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	International Normalization Ratio
ip	intraperitoneal
IPC	In-process control
IR	Infrared
IRB	Institutional Review Board
ISE	Integrated Summary of Efficacy
ISS	Integrated Summary of Safety
ITT	Intent-to-Treat
IU	International Units
iv	intravenous
Kd	elimination rate constant
kg	Kilogram
KGY	Kilogray
Km	Michaelis–Menten constant
Km	substrate concentration
lbs	Pounds
LD50	median lethal dose
LDH	Lactate Dehydrogenase
LDPE	Low Density Polyethylene
LOA	Letter of Access
LOCF	last observation carried forward
LOD	Limit of Detection
LOQ	Limit of Quantitation
LoQ	List of Questions
LT	Less than
MA	Marketing Authorisation
MAA	Marketing Authorisation Application
MAH	Marketing Authorisation holder

MedDRA	Medical Dictionary for Regulatory Activities
metHb	methaemoglobin
mg	Milligram
MI	mitotic index
min	Minute
mL	Milliliter
mmol	Millimole
MN	micronucleus
MS	Mass Spectrometry
MTX	methotrexate
NA	not applicable
NAD	Nicotinamide Adenine Dinucleotide
NADH	nicotinamide adenine dinucleotide hydride
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate hydride
NADT	total NAD (NAD++NADH)
NADT	total nicotinamide adenine dinucleotide
NBR	negative binomial regression
ND	Not detected
NDA	New Drug Application
NK	natural killer
NLT	Not less than
NMR	Nuclear Magnetic Resonance
NMT	Not more than
NOAEL	no observed adverse effect level
NP	Nurse Practitioner
NS	not significant
NTB	non-tumour bearing
OIL	sesame oil
OOS	Out of Specifications
OTC	over-the-counter
P	p-Value
PA	Physician’s Assistant
PAF	platelet activating factor
Pbo	placebo
PBS	phosphate buffered saline
PCV	packed cell volume
PE	Polyethylene
PGA	pyroglutamic acid
Ph. Eur.	European Pharmacopoeia
PI	Product information
PIL	Patient Information Leaflet
PK	pharmacokinetic
PNA	polynitroxyl albumin
PP	Per-Protocol
PPM	Parts per million
PRU	peripheral resistance units
PSUR	periodic safety update report
PT	preferred term
QOS	Quality Overall Summary
Ra	appearance rate
RBC	Red Blood Cell
Redox	Reduction
Retic.	Reticulocyte
RH	Relative Humidity
RMS	Reference Member State
RP	Restricted Part (or Closed Part) of a ASMF
RRT	Relative retention time

RSD	Relative standard deviation
SAE	Serious Adverse Event
SBS	short bowel syndrome
SCC	sickle cell crisis
SCD	Sickle cell disease
SCE	sister chromatid exchange
SCE	Summary of Clinical Efficacy
SCS	Summary of Clinical Safety
SD	standard deviation
SDH	succinate dehydrogenase
SEM	standard error of the mean
SME	Small Medium Enterprise
SmPC	Summary of Product Characteristics
SOC	system organ class
SOD	superoxide dismutase
SPC	Summary of Product Characteristics
SS	Homozygous
t1/2	half-life
TEAE	treatment-emergent adverse event
tid	three times a day
TLC	Thin layer chromatography
tpf	pressure-flow recovery time
ULN	upper limit of normal
US	United States
USP	United States Pharmacopoeia
UV	Ultraviolet
Vd	volume of distribution
Vmax	maximum elimination rate
WBC	white blood cell
WPBR	whole body protein breakdown rate
XRD	X-Ray Diffraction
µL	Microliter
µM	Micromole

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Emmaus Medical Europe Ltd submitted on 16 January 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Xyndari, through the centralised procedure falling within the Article 3(1) and point 4 of Annex I of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 January 2017.

Xyndari was designated as an orphan medicinal product EU/3/12/1011 on 4 July 2012 in the following condition: Treatment of sickle cell disease.

The applicant applied for the following indication: Xyndari is indicated for the treatment of sickle cell disease (SCD).

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 study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0003/2018 from 4 January 2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0003/2018 was not yet completed as certain measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Protocol assistance

The applicant did not seek Protocol assistance at the CHMP.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Milena Stain Co-Rapporteur: Nithyanandan Nagercoil

The application was received by the EMA on	16 January 2018
The procedure started on	1 March 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	22 May 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	23 May 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	4 June 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	26 June 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	26 November 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	08 January 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	17 January 2019
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	31 January 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	25 March 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	11 April 2019
The major objections were addressed by the applicant during an oral explanation before the CHMP during the meeting on	25 April 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Xyndari on	29 May 2019

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Xyndari (glutamine) is intended for the prevention of sickle cell crises in adults and children 5 years and older suffering from Sickle Cell Disease (SCD).

SCD is a genetic disorder. In patients with SCD haemoglobin is altered causing the deformation of erythrocytes and subsequent vaso-occlusive events and chronic haemolytic anaemia. The main manifestations of SCD include painful crises including chest, back and joints, organ damage and varying degrees of anaemia and related symptoms.

2.1.2. Epidemiology

SCD is a heritable disease mainly prevalent in sub-Saharan Africa due to a protective effect for malaria of the heterozygous form. The numbers of patients with SCD however is steadily increasing in the EU due to migration. The prevalence in the EU is currently estimated to be ≤ 2.1 per 10000 individuals.

2.1.3. Aetiology and pathogenesis

SCD is an autosomal recessive trait determined by combinations of two abnormal alleles of the beta globin gene among which at least one carries the beta 6 glu-val mutation (HbS). The term sickle cell disease covers a group of genetic disorders which all present at least one haemoglobin S allele. Although these disorders vary in the second beta globin variant, they all result in abnormal polymerisation of haemoglobin.

The homozygous variant of HbSS is the most common form of SCD worldwide and in the EU (about 90% of SCD patients in the EU). The second most common form in the EU is sickle β-thalassemia (~10%) which can occur in two forms (Hb S/β+-thalassemia and Hb S/β°-thalassemia); rarer forms exist but not in Europe so far.

The mutation in the beta-chains alters the polymerisation of haemoglobin resulting in deformed erythrocytes under certain conditions. The deformation leads to a sickle shape resembling a crescent moon. The deformed erythrocytes exhibit also an increased adhesion resulting in increased interconnection and adhesion to the surface of the blood vessels causing vaso-occlusive events. The deformed erythrocytes are furthermore metabolised resulting in anaemia and related symptoms.

The deformation of erythrocytes has been related to increased oxidative stress and altered NAD/H redox potential.

2.1.4. Clinical presentation, diagnosis and prognosis

Clinical presentation

Symptoms of sickle cell disease vary between patients but are generally caused by intermittent vaso-occlusive events and chronic haemolytic anaemia.

Vaso-occlusive events result in tissue ischemia leading to acute and chronic pain mainly located in the chest, back, hands, feet and joints. These events cause also organ damage affecting any organ system including the bones, spleen, liver, brain, lungs, kidneys and joints. Acute chest syndrome is a major cause of mortality in SCD.

Often the earliest manifestation of SCD is dactylitis (pain and/or swelling of the hands or feet). In children, the spleen is of major concern as it is particularly vulnerable to infarction. The majority of untreated patients with SCD become functionally asplenic in early childhood, increasing their risk for certain types of bacterial infections.

Chronic haemolysis can result in varying degrees of anaemia, jaundice, cholelithiasis, delayed growth and sexual maturation. Individuals with the highest rates of haemolysis are predisposed to pulmonary artery hypertension, priapism, and leg ulcers.

Prognosis:

Life expectancy is decreased in patients with SCD to 42 - 53 years for men and 48 - 58 years for women. Vaso-occlusive events like cardiac arrest, thrombosis or stroke and organ failure are major causes of mortality in SCD.

Diagnosis:

Sickle cell disease usually manifests for the first time with symptoms and deformed erythrocytes in infants at the age of about 6 months when foetal haemoglobin is replaced by HbS. However, SCD can be diagnosed earlier via newborn screening or even genetic testing during pregnancy, which has been an established measure in the US and several European counties. Otherwise the diagnosis of SCD is established via molecular genetic testing to identification of significant quantities of HbS.

2.1.5. Management

The management of SCD includes mainly the treatment of clinical manifestations and preventive measures including early education of patients and parents. Early diagnosis allows early education and intervention before symptoms or organ damage manifest, drastically improving mortality in children over the last 20 years.

The treatment of clinical manifestations consists mainly of the management of pain episodes (hydration, anti-inflammatory agents, pain medication, massage etc). Fever and suspected infection is treated with appropriate antibiotics. Life-threatening or severe complications (e.g., severe acute chest syndrome or stroke) are often treated with red blood cell transfusion. Splenectomy may be necessary for splenic sequestration.

Preventive measures include maintaining hydration and avoid climate extremes or other unfavourable environmental factors. Chronic red blood cell transfusion is used in children at risk for stroke and individuals with pulmonary hypertension, chronic renal failure, recurrent acute chest syndrome, and severe end-organ damage. Hydroxyurea (HU) is approved for the prevention of recurrent painful vaso-occlusive crises. Stem cell transplantation may be an option in selected individuals. Other preventive measures include management of fevers, prophylactic antibiotics, immunisations, folic acid supplementation; and iron chelation therapy for those with iron overload.

An essential part of the management of SCD is periodic monitoring of significant parameter: comprehensive medical and social evaluation, mental health and neurocognitive assessment, routine dental care, annual CBC and reticulocyte count, assessment of iron status, liver and renal function tests, urinalysis, LDH and vitamin D level. It also includes annual transcranial Doppler to determine the risk of stroke in all children with Hb S/S and Hb S/β°-thalassemia and ophthalmologic evaluation in all with sickling disorders. Evaluation of organ damage is performed as indicated via chest x-ray, ECG, abdominal ultrasound, and iron overload. Due to the high frequency and severity of cardiopulmonary complications, patients are frequently monitored with an echocardiogram, pulmonary function tests, and sleep study.

Different environmental factors have been identified to increase SCC and should be avoided by patients with SCD, e.g. dehydration, extremes of temperature, physical exhaustion, extremely high altitude, recreational drugs with vasoconstrictive or cardiac stimulation effects and meperidine.

Close monitoring is required for women with SCD during pregnancy. An increased risk for preterm labour, thrombosis, infectious complications, and acute painful episodes has been reported during pregnancy.

About the product

Xyndari contains the active substance glutamine and is presented as 'powder in sachet' with each sachet containing 5 g powder for oral use.

The total daily dose is 10 grams, 20 grams, or 30 grams based on subject weight, divided in two doses.

The indication initially applied for is: 'Xyndari is indicated for the treatment of sickle cell disease'. Later in the procedure the Applicant applied for the following revised indication: 'Xyndari is indicated for the prevention of sickle cell crises in adults and children 5 years and older suffering from sickle cell disease'.

The pharmacotherapeutic group is: other alimentary tract and metabolism products, ATC code A16AA03

The mechanism of action of the amino acid glutamine in the prevention of sickle cell crises is not fully understood. Oxidative stress phenomena are involved in the pathophysiology of sickle cell disease. Sickle red blood cells (RBCs) are more susceptible to oxidative damage than normal RBCs, which may contribute to the chronic hemolysis and vaso-occlusive events associated with sickle cell disease.

Type of Application and aspects on development

- Legal basis

This marketing authorisation application for Xyndari (glutamine) is made according to Article 8(3) of the Directive 2001/83/EC as amended, a complete and independent application. It relies on studies conducted by the applicant and on relevant bibliographic references to support both nonclinical and clinical aspects.

The active substance in Xyndari is glutamine. Glutamine is a known active substance and has been licensed in the US for the treatment of short bowel disease in 2004 and for the treatment of sickle cell disease in 2017. Glutamine is a parenteral nutrition substrate and was licensed as such in many decentralised and national procedures.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as oral powder containing 5 g of glutamine as active substance.

The finished product contains no excipients.

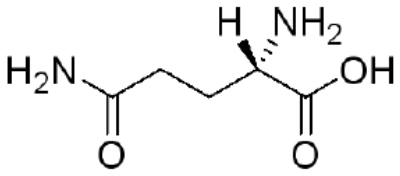
The product is available in paper/aluminium/polyethylene laminate sachets as described in section 6.5 of the SmPC.

2.2.2. Active substance

General information

The chemical name of active substance is glutamine corresponding to the molecular formula $C_5H_{10}N_2O_3$. It has a molecular mass of 146.14 g/mol and the following structure:

Figure 1: active substance structure



The chemical structure of active substance was elucidated by FTIR spectroscopy. In addition, the optical rotation results are presented to show the optical characteristics of the isomer. The structure of the active substance has been confirmed by comparison of IR spectra between the designated reference standard and three different lots of active substance. Since glutamine is an orthorhombic crystal, which does not exhibit polymorphism, the presented structure elucidation information was considered sufficient and no other technique has been used to confirm the chemical structure of the material.

The active substance is a white to off-white, free flowing crystalline, non-hygroscopic powder, soluble in water and practically insoluble in ethanol and in diethyl ether.

Glutamine exhibits stereoisomerism due to the presence of one chiral centre. The active substance manufacturer controls isomerisation by synthesizing the L-glutamine through a recombinant process, which precludes the formation of the D-glutamine. The naturally occurring form of glutamine is the L-isomer. Enantiomeric purity is controlled routinely by specific optical rotation in the active substance specifications.

L-Glutamine is not monographed in the European Pharmacopoeia (Ph. Eur.), however a monograph exists in the German Pharmacopoeia (DAB) and the United States Pharmacopoeia (USP).

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory, except for several minor concerns not affecting the benefit/risk of the product.

The active substance is obtained from a single manufacturer.

Glutamine is manufactured via a bacterial fermentation process using a bacterial strain, which exclusively expresses the L-enantiomer of glutamine. Satisfactory information regarding the composition of each medium used in the fermentation process, the antifoam agent, the sterilisation conditions and process applied to starting material and culture media has been presented. The endpoint indicators for the fermentation process have been clearly stated.
Following the fermentation in closed and sterilized systems using sugar as main raw material, the fermented broth containing the active substance is processed in the isolation step and further purified. The purification step consists of 8 steps illustrated in scheme 1: resin treatment, de-colorization, filtration, concentration, crystallisation, separation, drying and packing.

Figure 2: active substance manufacturing process

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

Powder flowability is considered a critical quality attribute of the active substance; critical physical properties of the active substance impacting powder flow is controlled in the specification according to fully justified acceptance limits. The analytical methods applied should be fully described and validated.

The active substance is packaged in a polyethylene (PE) bag which complies with the EC directive 2002/72/EC and EC 10/2011 as amended. The primary PE bag is placed into a secondary bag which may be placed in an appropriate shipping fiber drum.

Specification

The active substance specification is based on the German Pharmacopoeia (DAB) and includes tests for: description (visual), identification (DAB), assay (HPLC), related compounds (HPLC), state of solution (DAB), pH (DAB), specific rotation (DAB), chlorides (DAB), sulphates (DAB), iron (DAB), elemental impurities (DAB), loss on drying (DAB), sulphated ash (DAB), and particle size (Ph. Eur.).

At the time of opinion, a clarification was left outstanding by the Applicant whether this parameter should be included in the active substance specification of the Applicant as well if the Applicant applies a method different from that of the active substance manufacturer. In that case method description and validation data should be presented. This point is considered a minor unresolved quality issue at the time of Opinion having no impact on the Benefit/Risk ratio of the product.

As the active substance is a fermentation product, ICH Q3A thresholds as well as Ph. Eur. general monograph Substances for Pharmaceutical Use (2034) do not apply. Impurities are controlled by a limit that is justified by batch and stability data, and qualified with respect to safety.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data (n=31 commercial scale) of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 6 commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. Photostability testing following the ICH guideline Q1B was performed on one batch.

The following parameters were tested*:* state of solution (transmittance in HCl and H2O), loss on drying, pH, assay, specific rotation (in HCl and H_2O), ammonium and related substances. The parameters tested are the same as for release. The analytical methods used were the same as for release and were stability indicating.

All tested parameters were within the specifications.

Results on stress conditions: acidic (0.1 N HCl, 24 h), basic (0.1 N NaOH, 24 h), oxidative (3% hydrogen peroxide, 24 h), thermal stress (80°C, 72 h), and light stress (765 w/m2, 8 h) were also provided on one batch. Stress studies showed that glutamine is sensitive to acidic, basic and oxidative conditions.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 36 months in the proposed container closure system.

2.2.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is presented as a single-use sealed sachet containing 5 g of glutamine powder. The finished product is composed of the neat active substance and it contains no excipients.

The finished product is intended for oral use in increments of 5 grams with an upper limit of 30 g/day, administered twice daily. The glutamine powder should be mixed immediately before administration with 240 ml of cold or room temperature beverage, such as water, milk, or apple juice, or 113 grams to 170 grams of food such as yogurt or applesauce. Complete dissolution is not required prior to administration.

Table 1: composition of finished product

Ingredient	**Reference**	**Amount (per unit sachet)**	**Function**
Glutamine	-	5 g	Active substance

Factors incorporated into the design of the oral glutamine sachets included convenience of handling and ease of use by the patients. To maximize handling convenience and ease of dosing, the finished product is packaged in single-unit dose sachets containing 5 grams of glutamine.

Xyndari is compositionally the same as the pharmaceutical product, NutreStore (oral glutamine sachets), that the Applicant markets in the United States for the treatment of short bowel syndrome. The two products consist of 5 grams of glutamine packaged in a sachet of identical materials and methods of manufacture.

Oral glutamine powder is soluble in water.

Particle size of the active substance is controlled during glutamine manufacture by pulverization, followed by sieving to remove lumps. The active substance manufacturer demonstrated consistent control of the particle size and powder flow that is important for manufacturability. In addition, particle size is controlled in the active substance specifications applied by the finished product manufacturer.

The manufacturing process of the finished product is simple. No additional processing besides packaging is performed on the bulk glutamine active substance prior to packaging into the unit dose sachets. No process development was performed.

The formulation used during pivotal clinical studies is the same as that intended for marketing.

To confirm compatibility of oral glutamine with the dosing instructions, a study was conducted using water, apple juice, milk, apple sauce, and yogurt.

A reconstitution study was performed to determine the stability of glutamine when dissolved in water. This study demonstrated that glutamine did not degrade within 6 hours of reconstitution in water.

The primary packaging is paper/aluminium/polyethylene laminate sachet with side-tear notches constructed of a pre-printed paper-foil-plastic laminate. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The finished product manufacturing process consists of filling of sachets with the active substance. The process is divided into 5 steps and it is considered to be a standard manufacturing process.

Sachet filling and sealing are considered a critical part of the process. Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (visual), identification (Ph. Eur.), assay of glutamine (HPLC), related substances (HPLC), mass variation (Ph. Eur.), loss on drying (Ph. Eur.) and microbial limit tests (Ph. Eur.).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. 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 and the presented batch data it can be concluded that the following elemental impurities: As, Hg, Pb, and Cd are measured on the active substance as part of release testing and are fully controlled. The information on the control of elemental impurities is satisfactory.

Batch analysis results are provided for 15 commercial batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Stability data from 1 commercial scale batch of finished product stored for up to 12 months under long term conditions (25 ºC / 60% RH) and data from 3 commercial scale batches stored for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of the medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

In addition, stability data on two clinical lots manufactured for up to 48 months at the 25 °C/60% RH long term storage condition and 6 months at the accelerated storage condition of 40 °C/75% RH were provided with 3 attributes tested according to proposed specifications: appearance, loss on drying, and microbial limits test. However, in those studies assay and related substances were tested by titration and thin layer chromatography (TLC) as specified in compendial monographs. The Applicant changed their control strategy after these stability studies were completed; and thus, has presented limited stability data on the assay and related impurities attributes using the HPLC method listed in the dossier.

Despite different analytical procedure used for testing of the clinical batches, the assay by titration results for these lots were stable while on stability, and as such support the stability of the product at long-term and accelerate conditions. Also, additional supportive stability data for 18 months for one clinical batch at

controlled room temperature provides evidence of no change to assay and related impurities levels when tested by current HPLC method.

Samples were tested for: description/appearance, identification (Ph. Eur.), assay of glutamine (HPLC), related substances (HPLC), loss on drying (Ph. Eur.) and microbial limit tests (Ph. Eur.). The analytical procedures used are stability indicating. The methods and acceptance criteria for the attributes of appearance, loss on drying, and microbial limits were the same as those used for release. During development stability studies, for assay and related substances used the titration and a thin layer chromatography methods, respectively, specified in the USP monograph instead of the proposed HPLC method. Conformance with the attributes tested by the HPLC method was assessed and found acceptable.

In all stability studies conducted to date for both the clinical and commercial lots, no attribute has shown any change from initial results outside of method variability and no trends have been observed.

Photostability data conducted on the active substance exposed directly to light per the conditions described in the ICH guidance "Q1B: Stability Testing: Photostability Testing of New Drug Substances and Products" showed no degradation. Since the finished product is identical to the active substance and per the decision tree in the guidance, the Applicant has not conducted photostability on the finished product which was acceptable. In addition, the finished product is packaged in single-use foil sachets that protect the contents from light.

Based on available stability data, the proposed shelf-life of 2 years as stated in the SmPC (section 6.3) is acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, and pharmaceutical 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.

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. Considering that the CHMP opinion does not conclude in a recommendation for a marketing authorization these issues are not further pursued

2.2.5. 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. Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendations for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical information is derived entirely from pharmacodynamic, pharmacokinetic, and toxicology data in the scientific literature and additional toxicology studies provided by the original NutreStore glutamine supplier.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The Applicant provided a total of 10 *in vitro/ex-vivo* nonclinical studies, of which 3 are peer-reviewed scientific journal articles and 7 are nonclinical pharmacology studies that the Applicant has conducted and have been published.

Table 2: Overview of pharmacodynamic studies with glutamine in establishing the pathogenesis of SCD

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Lachant et al, 1983
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Lachant and Tanaka, 1986
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Zerez et al, 1987
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Zerez et al, 1988
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Lachant et al, 1989
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Zerez et al, 1990a
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Zerez et al, 1994
	In vitro Human erythrocytes	NA	Harbor- UCLA Medical Center	Niihara et al, 1997
	Human erythrocytes	Oral	Harbor- UCLA Medical Center	Niihara et al, 2005
	Human erythrocytes	Ex vivo	NR	Kaul et al, 2006

NA = not applicable; NR = not reported.

Regarding the relevance of oxidative stress in the context of SCD, the Applicant discussed the following endpoints:

- oxidative damage of sickle RBCs
- adherence of sickle RBCs to vascular endothelium

The information provided to substantiate the role of oxidative damage of sickle RBCs is essentially based on the following publications: Zerez et al., 1988, showed that compared to normal RBCs sickle RBCs have an increased NAD content, while NADH (together with NADPH) content remains unchanged. Thus, the

NADH/NAD ratio in sickle RBCs is decreased indicating a reduced redox potential of diseased RBCs. Another paper from the same author (Zerez et al. 1987), showed impaired NAD synthesis for sickle RBCs, while NAD degradation remained comparable to normal RBCs.

The Applicant provided literature establishing a causal link between the abnormal NAD content and a higher concentration of 2,3-diphosphoglycerate as well as an impaired methaemoglobin reductase activity in sickle RBC in comparison to normal RBC.

Several publications elaborating on plasma and RBC glutamine levels in SCD patients as well as on glutamine transport were provided. Zerez et al., 1994, demonstrated that glutamine levels are elevated in the plasma as well as in RBC of SCD patients. Together with an increase in active glutamine transport as well as higher turnover of glutamine to glutamate identified in sickle RBC it was concluded that the higher glutamine concentration accounts for the higher NAD levels in sickle RBC (Niihara et al., 1997).

The information provided by the Applicant on the role of L-glutamine in context with endothelial adhesion of sickle RBC is limited to an in vitro assay with HUVECs involving RBC from patients treated with L-glutamine. The data quality of this study is poor and does not explain the mechanism underlying the supposed reduction of sickle RBC adhesion.

Non-clinical in vivo data in SCD models have not been generated, which is regarded as a weakness in the overall line of evidence.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were performed.

Safety pharmacology programme

No safety pharmacology studies were performed.

Pharmacodynamic drug interactions

The Applicant did not perform studies on pharmacodynamic drug interactions but provided a literature review on various substances that have been co-administered with glutamine but which are not considered primarily relevant for SCD patients.

2.3.3. Pharmacokinetics

No pharmacokinetic (PK) studies of glutamine were performed.

All information in the nonclinical dossier regarding the pharmacokinetic of glutamine was taken from published literature. Pharmacokinetic studies were described to be conducted in mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, piglets, sheep, and monkeys. These studies examined absorption, distribution, metabolism and excretion of glutamine administered *in vitro* and *in vivo* (orally, intravenously, and intraperitoneally).

Absorption

Type of Study	Test System Species/Strain/Gender	Method of Administration	Testing Facility	Study Reference
Absorption	Rat/Wistar/Male	Intestinal vascular perfusion or Intestinal luminal perfusion	NR	Hanson and Parsons, 1977
	Rat/Osborne-Mendel/Male	Intestinal luminal perfusion	NR	Windmueller and Spaeth, 1975
	Rat/Sprague-Dawley or Osborne-Mendel/Male	Intestinal vascular perfusion	NR	Windmueller and Spaeth, 1974
	Rat/NR/Male	Oral (force-fed)	NR	Peraino and Harper, 1962

A number of experiments used isolated rat intestinal segments to measure intestinal glutamine absorption. Results of these experiments showed that glutamine was absorbed rapidly from the lumen of the small intestine with more than 92% of radiolabelled glutamine taken up in the first 15 minutes (Windmueller and Spaeth, 1978; Windmueller, 1982). The greatest uptake of glutamine was seen in intestinal mucosal epithelial cells where 80% of glutamine was taken up and converted to metabolites 20 seconds after luminal infusion (Windmueller, 1982). Results of these studies also showed that the net tissue uptake of glutamine was concentration dependent and continuous at the rate of 10.8 to 11.1 mg/h (Windmueller and Spaeth, 1974; Windmueller, 1982).

Further studies indicated that the rate of glutamine uptake in isolated intestinal preparations derived from nonfasted animals was approximately 2 times the rate of glutamine uptake in those segments derived from fasted rats. In *in vivo* rat experiments where glutamine was administered via oral force-feeding, the systemic maximum concentration of drug in plasma (Cmax) of approximately 3.8 µM/mL was observed at 1.5 hours. Additionally, glutamine plasma levels quickly declined, indicating that it was rapidly distributed into organs and tissues (Peraino and Harper, 1962).

Distribution

Type of Study	Test System Species/Strain/Gender	Method of Administration	Testing Facility	Study Reference
Distribution	Mice/NR/NR Rat/NR/NR	iv	NR	Schwerin et al, 1950
	Rat/Sprague-Dawley/Male	Oral or ip	NR	Tigerman and MacVicar, 1950
	Rat/Sprague-Dawley/Male	iv	NR	Wu et al, 2000
	Rat/Wistar/female	iv	NR	Dierks-Ventling et al, 1971
	Rat/Osborne-Mendel/Male	iv	NR	Berenbom and White, 1949

Numerous studies showed that glutamine was distributed to many different organs and tissues (heart, lung, liver, kidney, blood, pancreas, muscle, brain, spleen and adrenal glands) after oral, intraperitoneal, or intravenous administration in mice and rats. However, most endogenous glutamine is synthesised and stored in skeletal muscle, where free glutamine is released to other tissues (Souba *et al,* 1990).

Exogenously administered glutamine was distributed to multiple organs and tissues, however, its distribution varied across species (ie, rat and mouse: Berenbom and White, 1949; Schwerin *et al,* 1950; Tigerman and MacVicar, 1950; Wu *et al,* 2000).

Schwerin *et al* (1950) administered a bolus tail vein injection of 7% glutamine (1200 to 1300 mg/kg) to mice, and rats. At baseline (0 minutes), 10, 15, 20 (rats only), 30, 45 (mice only), and 60 minutes (rats only) after injection of glutamine, animals were sacrificed and brain, liver, kidney, muscle (rats only), and blood were analysed for glutamine content. In rat brain, glutamine levels were significantly higher at 15, 20, and 30 minutes after injection ($P < 0.05$). Rat liver had significant increases in glutamine concentrations (compared with baseline values) at 10, 15, 20, and 30 minutes after injection ($P < 0.05$). Rat kidney and blood glutamine concentrations were also significantly higher than baseline levels at 15 and 30 minutes after injection ($P < 0.05$). Rat muscle glutamine levels did not significantly change in comparison with baseline levels. In mice, liver and kidney glutamine levels did not significantly change in comparison with baseline values; however, brain concentrations at 15 minutes post injection and blood concentrations at 15, 30, and 45 minutes post injection were significantly higher than baseline ($P < 0.05$).

Metabolism

Type of Study	Test System Species/Strain/Gender	Method of Administration	Testing Facility	Study Reference
Metabolism	Rat/Osborne-Mendel/Male	Intestinal luminal perfusion	NR	Windmueller and Spaeth, 1980
	Rat/Osborne-Mendel/Male	Intestinal arterial perfusion	NR	Windmueller and Spaeth, 1978
	Rat/Sprague-Dawley or Osborne-Mendel/Male	Intestinal vascular perfusion	NR	Windmueller and Spaeth, 1974
	Rat/NR/Male	Oral (force-fed)	NR	Peraino and Harper, 1962
	Rat/Sprague-Dawley/Male	iv Vascular perfusion of nonhepatic splanchnic organs	NR	Matsutaka et al, 1973
	Piglets/Cross-bred/Female	Enteral or iv infusion	NR	Stoll et al, 1999
	Sheep/Cross-bred/Female	Iv infusion	NR	Heitmann and Bergman, 1978
	Rats/Wistar/Male	In vitro incubation of lymphocytes	NR	Ardawi and Newsholme, 1983

Glutamine is converted to α-ketoglutarate, which is an integral part of the citric acid cycle. Numerous important metabolic products are derived from glutamine metabolism (Neu, 2001).

Endogenous glutamine plays a major role in the inter-organ transport of nitrogen, carbon, and energy within the body (Windmueller, 1982; Newsholme *et al,* 2003).

The intestine is a major site of glutamine metabolism where glutamine is absorbed luminally and metabolised within enterocytes (Windmueller and Spaeth, 1974). Some studies have calculated that the intestine of a 280g rat ingesting a 20% protein diet can metabolize a total of 3.6 mmol (526 mg) of glutamine per day (Windmueller, 1982).

A study examined endogenous glutamine metabolism in the gastrointestinal tract assessed by the relative activities of glutaminase and glutamine synthetase in rats (James *et al,* 1998). Results showed that glutaminase activity was highest in the mucosa of the small intestine, whereas mouth and oesophagus had very low glutaminase activity. Glutamine synthetase activity was seen mainly in the lower part of the stomach and was low in the small intestine, mouth and oesophagus. The investigators concluded that the mucosa of the small intestine had the highest capacity for glutamine breakdown, but the lowest capacity for glutamine synthesis and, thus, the mucosa of the small intestine required an external source of glutamine (James *et al,* 1998).

Once glutamine has entered the bloodstream after intestinal absorption and distribution to tissues, it is metabolised into various products and acts as a substrate for protein synthesis, anabolic precursor for muscle growth, acid–base balance in the kidney, substrate for ureogenesis in the liver, inter-organ nitrogen transport, substrate for hepatic and renal gluconeogenesis, an oxidative fuel for intestine and cells of the immune system, precursor for neurotransmitter synthesis, precursor for nucleotide and nucleic acid synthesis, and precursor for glutathione production (Tapiero *et al,* 2002; Newsholme *et al,* 2003).

When exogenous glutamine is administered, the metabolic pathways and derivatives parallel those seen with endogenous glutamine.

As glutamine is metabolised, the majority of its intermediary metabolites are incorporated into other proteins or stored in tissues. However, the nitrogen from glutamine that is not stored or incorporated into proteins or tissues is generally converted into urea and excreted in the urine. In a rat experiment, 62% of glutamine's nitrogen was recovered as urinary urea after 24 hours; whereas, only 4.2% of the total nitrogen was recovered as ammonia (Berenbom and White, 1949).

In another study performed by Ardawi *et al*, glutamine could not be replaced by other amino acids or ammonia. At a concentration of 2 mM in incubation medium, arginine, asparagine, aspartate, glutamate, glycine, histidine, proline, serine, or ammonium chloride stimulated [3H]thymidine incorporation to only 10% to 20% of that produced by the same concentration of glutamine (Ardawi and Newsholme, 1983).

Excretion

Type of Study	Test System Species/Strain/Gender	Method of Administration	Testing Facility	Study Reference
Excretion	Rat/Osborne-Mendel/Male	iv	NR	Berenbom and White, 1949

The majority of the glutamine intermediary metabolites are incorporated into other proteins or stored in tissues.

However, the nitrogen from glutamine that is not stored or incorporated into other molecules is generally converted into urea and excreted in the urine (Berenbom and White, 1949). The majority of the carbon from glutamine is ultimately metabolised to carbon dioxide (Windmueller and Spaeth, 1974).

One study performed by Berenbom et al showed that when the glutamine amide nitrogen molecule was radiolabeled (Nitrogen-15 [15N]) and injected into the jugular vein of rats, it was rapidly cleared from the circulation, distributed to the liver and kidneys, and converted and excreted as urea (Berenbom and White, 1949). After 24 hours, only 5.1% of the total 15N was found in the liver, 2.4% in the blood, and 0.8% in the kidneys. These results indicate that glutamine is distributed to the liver and kidneys, but rapidly metabolised and excreted in the urine (Berenbom and White, 1949).

Male Osborne-Mendel rats were administered a jugular vein injection of 4.75 mg of amide [15N]-glutamine (22 mg/kg or 132 mg/m2). At 15 minutes, and 1, 6, and 24 hours, 4 animals were sacrificed and blood, liver, kidneys, and urine were collected and pooled. The distribution of 15N in these 4 areas was determined. Results showed that glutamine was rapidly removed from the blood, converted to urea, and excreted in the urine.

In 24 hours, 66% of the 15N was recovered in the urine, 94% (62% of the total) of which was urinary urea. After 24 hours, only 4.2% of the total 15N was recovered as ammonia.

Pharmacokinetic drug interactions

No nonclinical studies evaluating the potential for a PK interaction between glutamine and drugs commonly used by patients with SCD (e.g., hydroxycarbamide and opioids) were performed.

However, the Applicant submitted published nonclinical studies on the effects of combining glutamine with other agents such as:

- **indomethacin** (Arndt *et al,* 1999) Glutamine. Attenuates Leukocyte-Endothelial Cell Adhesion in Indomethacin-Induced Intestinal Inflammation in the Rat
- **cyclosporine** (Zhang *et al,* 1995) Glutamine Reduces Bacterial Translocation after Small Bowel Transplantation in Cyclosporine-Treated Rats
- **methotrexate** (Fox *et al,* 1988) Effect of a Glutamine-Supplemented Enteral Diet on Methotrexate Induced Enterocolitis in Rats
- **5-fluorouracil** (Jacobs *et al,* 1987) Disparate Effects of 5-Flourouracil on the ileum and colon of enterally fed rats with protection by dietary glutamine
- **dexamethasone** (Boza *et al,* 2001b) Effect of Glutamine Supplementation of the Diet on Tissue Protein Synthesis Rate of Glucocorticoid-Treated Rats

In these studies, glutamine treatment did not cause any damaging effects.

2.3.4. Toxicology

No toxicity studies were performed by the Applicant. The information presented on the toxicological evaluation of glutamine to support the marketing application is literature based.

Single dose toxicity

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (g/kg)	Noteworthy Findings	Study Number
Mouse	po (vehicle not indicated)	NR	NR	NR	LD_{50}: 21.7 in males and 20.3 in females	No other information provided	Kyowa Hakko, 2001
Mouse	iv (vehicle not indicated)	NR	NR	NR	LD_{50}: 4.5 in both sexes	No other information provided	Kyowa Hakko, 2001
Rat	po (vehicle not indicated)	NR	NR	NR	LD_{50}: 7.5 in males and 10.5 in females	No other information provided	Kyowa Hakko, 2001
Rabbit	po (vehicle not indicated)	NR	NR	NR	LD_{50}: 18.8 in males	No other information provided	Kyowa Hakko, 2001

LD_{50} = medial lethal dose; NR = not reported; po = oral; iv = intravenous.

Single-dose toxicology studies have been conducted in mice, rats, and rabbits that received glutamine via oral and iv routes (Kyowa Toxicity Data of glutamine). Limited information on the methods of analysis in these studies was available because full study reports could not be obtained.

The oral median LD50 was 7.5 g/kg (45 g/m^2) in male rats and 10.5 g/kg (63 g/m^2) in female rats, whereas, it was 21.7 g/kg (65.1 g/m^2) in male mice and 20.3 g/kg (60.9 g/m^2) in female mice. The male rabbit oral LD50 was 18.8 g/kg (225.6 g/m^2), which was similar to the mouse LD50 based on mg/kg, but less sensitive based on a mg/m^2 basis, which suggested that mice and rats are more sensitive to the effects of glutamine

than rabbits. Although the oral LD50 for glutamine was not determined in female rabbits, the LD50 has been determined in the more sensitive species, mice and rats. However, the glutamine LD50 was considerably lower (approximately 4 times) when given intravenously as compared with the oral LD50 in both male and female mice, as a result of direct systemic administration.

The oral LD50 values represent approximately 2 to 10 times (45 g/m^2 to 225.6 g/m^2, respectively) the currently proposed total daily dose of 0.6 g/kg/day or 22.2 g/m^2.

Repeat dose toxicity

Safety Evaluation of N-Acetyl-L-glutamine Aluminum Complex Subacute Toxicity Experiments in Rats

Species/Strain: Rat/Sprague-Dawley Initial Age: 6 weeks Date of First Dose: 1974	Duration of Dosing: 30 days Duration of Post-dose: None Method of Administration: Oral gavage Vehicle/Formulation: 5% aqueous gum arabic solution for test article and distilled water for control animals	Study No. Oguro et al, 1974a Location in CTD: Vol. Module 2.6.6 Section 2.6.6.3 GLP Compliance: No

Kyowa Hakko conducted a 30-day repeat-dose oral gavage toxicity study with glutamine and KW-110 in Sprague-Dawley rats (10 animals/sex/group). In this study, glutamine was used as a comparator product.

Glutamine was administered to rats (10 animals/sex/group) by daily oral gavage at doses of 4, 6, and 10 g/kg/day (24, 36, and 60 g/m2/day) over 30 consecutive days (6 days a week). No glutamine-related clinical signs were noted throughout the study. Three animals from the 10 g/kg/day (60 g/m2/day) group died shortly after dosing: one male each on Day 14 and Day 15, and one female on Day 19. The report concluded that, given the high dose volume used, the deaths were likely attributable to complications from the technical procedures.

Body weight and food consumption were slightly reduced (7% and 11%, respectively) in male rats given 10 g/kg/day (60 g/m2/day) glutamine when compared with control rats given distilled water (Oguro *et al,* 1974a). No significant haematology changes were observed in any glutamine-treated animals. However, blood chemistry results showed that female rats administered 10 g/kg/day (60 g/m2/day) glutamine had statistically significantly higher blood glucose (21%) when compared with that of controls. No other significant changes in blood chemistry were noted. Urinalysis results showed that male control rats and animals administered the 4.0 g/kg/day (24 g/m2/day) dose were positive (qualified as a small amount) for urine occult blood.

Most of the absolute organ weights in glutamine-treated animals were unaffected by treatment with glutamine. Reduction of spleen weight (25%) and increased adrenal weights (26%) were observed at 10 g/kg/day (60 g/m2/day) in males, and increased liver weight (28%) was observed in females at the same dose level. Relative organ weights (relative to body weight) were reported to be significantly increased in males at 10 g/kg/day (60 g/m2/day) in a number of organs (heart, liver, kidneys, brain, adrenal, spleen, and sex organs), but were most likely related to the slightly reduced body weight observed in this treatment group (Oguro et al, 1974a). Relative organ weights were generally unaffected in females (no body weight changes) with the exception of lung, liver, and kidney at 10 g/kg/day glutamine (60 g/m2/day). The slight

changes observed in a few organs were likely related to the limited number of animals used (only 5 animals/sex/group, a subset of the original 10 animals/sex/group), and biological variability. In addition, the lack of dose response and consistency in the organs affected in males versus females, and the relatively small magnitude of the changes suggest that these changes are non-adverse and not toxicologically significant (Oguro *et al,* 1974a).

However, except for stomach catarrh, the incidence in the findings was comparable for animals treated with distilled water. It was concluded that given the nature of the changes, their prevalence and severity, and the absence of any dose response, most of the findings reported were not adverse or toxicologically significant (Oguro *et al,* 1974a).

Safety evaluation of N-Acetyl-L-glutamine Aluminum Complex - Chronic Toxicity in Rats

Species/Strain: Rat/Sprague-Dawley	Duration of Dosing: 180 days	Study No. Oguro et al, 1974b
Initial Age: 6 weeks	Duration of Post-dose: 30 days (subgroup)	Location in CTD: Vol. Module 2.6.6 Section 2.6.6.3
Date of First Dose: 1974	Method of Administration: Oral gavage	
	Vehicle/Formulation: 5% aqueous gum arabic solution at 50% concentration for GM, distilled water for control animals	
		GLP Compliance: No

A chronic repeat-dose toxicology study of glutamine was conducted by the Technical Research Laboratory, Hofu Plant of Kyowa Hakko.

In the 6-month study, glutamine and KW-110 were administered by oral gavage to rats (5 animals/sex/group) over a 180-day period (Oguro et al, 1974b). Satellite groups (3 animals/sex/group) were added to the study design, and animals in the groups were euthanised after 3 months of administration. Another set of animals (3 animals/sex/group) was dosed for 6 months, followed by a 30-day recovery period to examine the potential reversibility of the effects observed after 6 months of dosing.

In this study, glutamine was used as a comparator product.

Glutamine was administered to rats (3 to 5 animals/sex/group) by daily oral gavage at doses of 2 and 4 g/kg/day (12 and 24 g/m2/day) over 3 or 6 consecutive months (6 days a week).

In general, subchronic or chronic administration (3 or 6 months, respectively) of glutamine induced changes similar to those seen in the 30-day repeated-dose toxicology studies, namely slightly decreased food intake (approximately 8% to 9%), but with minimal effect on body weight (< 5%). It was concluded that the food consumption and body weight effects were likely attributable to complications from the technical procedures given the high dose volume used. However, the same dose volumes were used for control animals, and accordingly, a treatment-related effect cannot be excluded (Oguro et al, 1974b).

3-Month Interim Sacrifice Results**:** No treatment-related clinical signs were reported.

At 4 g/kg/day (24 g/m2/day) glutamine, significantly lower haematocrit (4% vs control; $P < 0.01$) and relative bands (1.7% vs control; $P < 0.01$) were noted. No toxicologically-significant clinical chemistry or urinalysis findings were noted in glutamine-treated animals (Oguro et al, 1974b). Relative organ weight (mg/100 g body weight) results indicated that after 3 months of glutamine dosing, male rats had increased relative liver weight (approximately 14%) at 2 g/kg/day (12 g/m2/day) and increased relative lung (approximately 9%) and adrenals (approximately 46% for right adrenals and 32% for left adrenals) weights at 4 g/kg/day (24 g/m2/day). Female rats had significantly increased relative heart weight (approximately 15%) at 4 g/kg/day glutamine (24 g/m2/day). These organ weight changes did not result in histopathological changes.

6-Month Results No mortality and no treatment-related clinical signs were noted during the study. Haematology results revealed that red blood cell (RBC), haematocrit values (reduced by approximately 11%), and white blood cell (WBC) counts were significantly lower in males treated at 2 and 4 g/kg/day glutamine (12 or 24 g/m2/day) when compared with controls. Females treated with 2 g/kg/day glutamine (12 g/m2/day) showed increased WBC counts when compared with controls, but such an effect was not observed at the highest dose level. At 4 g/kg/day glutamine (24 g/m2/day), increased platelet counts were also noted in males, and significantly higher RBC counts and lower platelet counts were noted in females. Parameter variations were not the same between males and females and were not always dose dependent, which suggests that changes were not adverse or toxicologically significant (Oguro *et al,* 1974b). Blood chemistry analysis results did not indicate treatment-related effects (Oguro *et al,* 1974b). Urinalysis results showed that after 6 months, female rats administered 4 g/kg/day glutamine (24 g/m2/day) had significantly higher urine volumes (+ 3.2 mL/16 hours) and lower urine protein concentrations (-66.0 mg/dL) than controls. Male rats treated with 2 g/kg/day glutamine (12 g/m2/day) were positive for urine occult blood (qualified as a small amount) at the 6-month point, but this effect was not observed at the higher dose level, suggesting that it is most likely incidental (Oguro *et al,* 1974b). No treatment-related effects were noted in organ weights after 6 months.

30-Day Recovery Group Results The differences in food consumption (approximately 9%) and body weight (approximately 6%) for females were still present during the recovery period. However, this finding may be related to the small number of animals used in the recovery phase of the study (ie, 3 recovery animals/sex/group). At the end of the 30-day recovery period, male rats that had received the 4 g/kg/day (24 g/m2/day) dose showed significantly higher relative lymphocyte counts and lower relative bands that were not observed during the treatment period. These results suggest normal biological variation. Female rats administered 4 g/kg/day glutamine (24 g/m2/day) had lower haematocrit values than the distilled water controls. The change in haematocrit (approximately 6% lower vs controls) was not observed during the treatment period, which indicates that it was related to the small number of animals that were used in this study (Oguro *et al,* 1974b). Female rats that received 4 g/kg/day glutamine (24 g/m2/day) also had significantly lower serum sodium concentrations when compared with those in control animals. This change, however, was not observed at the end of the treatment period, indicating that it was related to normal biological variation (Oguro *et al,* 1974b).

Thirteen-week Oral Toxicity Study of L-glutamine in Rats

Species/Strain: Rat/Sprague-Dawley	Duration of Dosing: 13 weeks	Study No. Tsubuku et al, 2004
Initial Age: 6 weeks at initial dose	Duration of Post-dose: 5 weeks	Location in CTD: Vol. Module 2.6.6 Section 2.6.6.3
Date of First Dose: NR	Method of Administration: Diet supplement	
		GLP Compliance: GLP Standards for Safety Studies on Drugs and Guidelines for Toxicity Studies Required for Applications for Approval to Manufacture Drugs
	Vehicle/Formulation: NR	

In a 13-week study, 6-week old male and female rats (N = 48) were randomised to 1 of 4 treatment groups: control diet (Group I), glutamine-supplemented diet at 1.25% (w/w diet; Group II), 2.5% (w/w diet; Group III), or 5.0% (w/w diet; Group IV). After 13 weeks of treatment, 6 control rats (Group I) and six 5.0% glutamine-supplemented rats (Group IV) were chosen to enter a 5-week recovery period where only a standard diet was administered. All other rats were sacrificed and evaluated for toxicity.

Throughout the administration and recovery period, no deaths occurred, and no noteworthy changes in diet consumption were noted. There was a slight trend towards decreased body weight gain in animals in Group IV (5% glutamine) relative to control animals; however, this effect was reversed during the 5-week recovery phase. Treatment with glutamine was not associated with any ophthalmology, gross pathology, or histopathologic changes.

The authors concluded that for both genders the definitive toxic level for glutamine is above 5.0% (3.379 g/kg/day [20.274 g/m2/day] for males and 4.026 g/kg/day [24.156 g/m2/day] for females) and the no observed adverse effect level (NOAEL) for glutamine was estimated at 1.25% (0.833 g/kg/day [4.998 g/m2/day] for males and 0.964 g/kg/day [5.784 g/m2/day] for females).

Based on the authors' interpretation, 1.25% glutamine is the NOAEL, which corresponds to an HED of approximately 0.134 g/kg/day to 0.156 g/kg/day (8 g/day to 9 g/day in a 60-kg human). However, data from this study indicate that the NOAEL is ≥ 5% glutamine corresponding to an HED of 33 g/day to 39 g/day.

Genotoxicity

Glutamine was not mutagenic in one *in vitro* bacterial mutagenicity assay and was considered devoid of clastogenicity *in vitro* or *in vivo* (Tavares *et al,* 1998; Oliveira *et al,* 2009; Wong *et al,* 2011; Xing and Na, 1996).

There are no *in vivo* studies for glutamine alone. The effect of glutamine and ascorbic acid on doxorubicin-induced clastogenicity effect was assessed in the *in vivo* chromosomal aberration study in rat bone marrow cells (Tavares *et al.,* 1998).

Several studies in the literature have reported that glutamine is able to prevent genetic damage in mice and rats exposed to genotoxic agents (Tavares *et al,* 1998; Mora *et al,* 2002; Oliveira *et al,* 2009; Wong *et al,* 2011; Turkez *et al,* 2012a; Turkez *et al,* 2012b; Oliveira *et al,* 2013). In terms of anti-genotoxicity, glutamine pretreatment was effective in reducing damage caused by chemotherapy treatment.

Carcinogenicity

Several nonclinical studies in the literature indicate that glutamine supplementation does not stimulate tumour growth (Bartlett *et al,* 1995; Buchman, 2001) and in some cases can inhibit tumour cell proliferation, (Yoshida *et al,* 1995; Liu *et al,* 2000) decrease tumour size, (Fahr *et al,* 1994; Klimberg *et al,* 1996; Klimberg and McClellan, 1996; Shewchuk *et al,* 1997; Liu *et al,* 2000) increase muscle protein synthesis, (Yoshida *et al,* 1995) and increase natural killer (NK) cell activity against malignant cells (Fahr *et al,* 1994; Klimberg *et al,* 1996; Klimberg and McClellan, 1996).

In a 2-year carcinogenicity studies in mice and rats with L-glutamic acid and MSG (Lmonosodium glutamate, and DL-monosodium glutamate), the results for L-glutamic acid and MSG were negative, up to 4% in the diet; negative results were obtained with MSG in Fischer rats up to 5% (up to 1982 mg/kg/day in males and 2311 mg/kg/day in females).

Reproduction Toxicity

The developmental and reproductive toxicity of glutamine has not been fully characterised.

A database search revealed that glutamine did not increase the prevalence of congenital malformations when tested in rabbits (Smith *et al,* 1965).

In a pilot teratology study in rabbits, doses of 0.15 g/kg/day glutamine administered orally over Gestational Days 7 to 12 did not lead to increased incidence of congenital malformations. Pregnant rabbits (5

animals/group) were treated orally with 0.15 g/kg/day (1800 mg/m2/day) glutamine during Gestation Day 7 through Gestation Day 12 (Smith *et al,* 1965). A total of 41 to 57 foetuses were examined, and the results showed that glutamine did not increase the prevalence of congenital malformations.

A study on the differentiation of whole embryonic rat eyes in organ culture examined the effects of glutamine (350 mg/L) in the culture media. Gestation Days 10, 11, and 12 embryos from pregnant Long-Evans rats were removed and cultured for 5 or 6 days. When compared with results from cultures in normal medium, results from cultures in the glutamine-supplemented medium showed marked increases in growth rate as evidenced by the number of mitotic figures present and the size of the eye anlagen. This acceleration of growth rate resulted in a number of morphological aberrations of the lens and the retina as compared with those from cultures in media containing 0.1 to 100 mg/L glutamine. Morphological aberrations can be seen in Armstrong and Elias, 1968.

Although no fertility studies have been reported in the literature, the evaluation of the reproductive organs in the rat 1-month and 6-month repeat-dose toxicity studies did not reveal any glutamine-related morphological changes (Oguro *et al,* 1974a; Oguro *et al,* 1974).

Toxicokinetic data

No toxicokinetic studies were performed nor found in the literature.

Local Tolerance

No local tolerance studies were performed by the Applicant nor found in the literature.

2.3.5. Ecotoxicity/environmental risk assessment

A justification for the absence of ERA studies was provided.

2.3.6. Discussion on non-clinical aspects

The majority of data available for the non-clinical assessment rely on the literature.

Pharmacology

In terms of primary pharmacology, the Applicant hypothesizes that glutamine supplementation may have a beneficial effect on sickle RBC morphology as well as on adhesion of sickle RBC to vascular endothelial cells. This assumption is based on a literature review indicating that the redox potential of sickle RBCs is impaired. The focus is thereby on the decreased NADH/NAD ratio, as a consequence of an increased NAD content observed in sickle RBCs as compared to normal RBCs. The comparatively high NAD levels have been causally linked to a high concentration of 2,3-diphosphoglycerate as well as an impaired methaemoglobin reductase activity. Altogether, these observations indicate higher susceptibility of sickle RBCs to oxidative damage as compared with normal RBCs. The Applicant's argumentation is comprehensible and the reported role of oxidative stress in the pathology of SCD is acknowledged. However, the line of evidence relies on general observations while direct, product/ substance-related proof of concept data is missing.

With regard to reduction of sickle RBC adhesion to vascular endothelial cells by glutamine, a publication demonstrating reduced adhesion of RBCs obtained from SCD patients treated with glutamine for several

weeks to HUVECs in vitro has been provided. The data, however, is scarce also for the inhibition of cellular adhesion by glutamine and important aspects such as the establishment of a dose-response relationship are missing. Moreover, the dependence of adhesion of RBC to vascular endothelial cells on specific biomarkers, e.g. adhesion molecules, plasma proteins, etc., is well characterized in the context with vaso-occlusive events in SCD in scientific literature, but no such data have been provided to support the suggested mode of action of glutamine.

Pharmacokinetics, Toxicology, Genotoxicity and Carcinogenicity

The Applicant did not conduct any own pharmacokinetic (PK) and toxicology studies of glutamine in support of the marketing application for the treatment of sickle cell disease (SCD). PK and toxicity data presented for this MAA derived from scientific literature data. However, the broad human use of commercially available glutamine and the known PK, safety and toxicity profile of the amino acid glutamine support the provided information and justify the lack of further PK and toxicity studies with Xyndari.

Reproduction toxicity

Dedicated animal reproduction studies were not conducted with Xyndari. Although developmental and reproductive toxicity are not covered according to the current standards, this is not considered to be of concern due to the broad data basis and detailed knowledge provided.

Local tolerance

Local tolerance studies were not conducted by the Applicant nor found in the literature. However, as glutamine is given orally, local gastrointestinal tolerance has been assessed in the nonclinical and clinical studies. This is acceptable, as widespread human use of commercially-available glutamine and the established safety of the pharmacological profile of glutamine supersedes nonclinical safety data.

Environmental risk assessment

A justification for the absence of a full environmental risk assessment has been presented and considered acceptable.

Because of the background knowledge and experience of the compound, it is not justifiable to ask for further non-clinical toxicity studies for the purposes of supporting this application.

In summary, although possible mechanism of action of glutamine in the context of oxidative stress in SCD has been provided, direct non-clinical evidence for this mechanism is lacking.

2.3.7. Conclusion on the non-clinical aspects

The evidence for a beneficial effect of glutamine on oxidative damage of sickle RBC as well as on vaso-occlusion by adhesion of RBC to endothelial cells provided by the Applicant is mainly based on general, literature-based observations. The proposed mode of action, i.e. the prevention of sickling by reducing the oxidative damage of sickle RBC, was not substantiated by studies in existing *in vivo* models for SCD or in vitro assays. In addition, the proposed inhibitory effect of L-glutamine on the adhesion of RBC to vascular endothelial cells could not be convincingly demonstrated in *in vitro* studies. This is a weakness in the line of evidence for the MoA in SCD and the pharmacologic effect of glutamine administration in sickle cells disease can therefore exclusively be evaluated on the clinical level.

Overall, the non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity or carcinogenic potential.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

Table 1. Listing of All Clinical Studies

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PD	Preliminary study no. 1	5.3.2.3	To measure rates of active RBC glutamine transport, glutamine transport kinetics with K_m and V_{max}, and plasma, and RBC levels of glutamine and glutamate	Open-label, controlled	NA[a]	Glutamine transport SCD: 11 high RTC: 6 healthy subjects: 7	Subjects with sickle cell anemia (homozygous Hb SS), subjects with reticulocytosis, healthy subjects	NA	Completed; Journal Article, (Niihara,1997)
						Glutamine transport kinetics SCD: 8 high RTC: 6 healthy subjects: 8			
						Plasma/RBC glutamine, glutamate levels SCD: 6 high RTC: 5 healthy subjects: 7			
PD	8288	5.3.4.2	To evaluate the effect of L-glutamine treatment on total NAD, NADH, and NAD redox potential of sickle RBCs, as well as hematologic parameters, subjective clinical response, and safety of L-glutamine supplementation	Pilot study, open-label, uncontrolled	L-glutamine 10 g/day; tid; Oral	7	Subjects with sickle cell anemia (homozygous Hb SS)	4 weeks	Completed; Journal Article (Niihara, 1998)

PD	10779	5.3.4.2	To evaluate the effect of L-glutamine treatment on the consistent improvement in the patients' subjective perception of clinical status (especially energy levels), safety, and exercise endurance	Open-label, controlled	L-glutamine 30 g/day (divided in 2 doses), control; bid; Oral	L-glutamine: 14 control: 5	Subjects with sickle cell anemia (homozygous Hb SS) or healthy subjects	12 weeks	Completed; Summary Report
Efficacy and Safety	10511	5.3.5.1	To evaluate the effect of L-glutamine treatment on exercise endurance, breath by breath exercise response, the incidence of painful crises, level of chronic pain, amount of daily requirement of narcotics, and safety	Phase 2, prospective, randomized, double-blind, placebo-controlled, parallel-group	L-glutamine 0.3 g/kg/day or placebo; bid; Oral	L-glutamine: 5 placebo: 10	Subjects with sickle cell anemia or sickle β^0-thalassemia	12 weeks	Terminated; Summary Report
PD, Efficacy and Safety	8775	5.3.5.1	To evaluate the effect of L-glutamine treatment on total NAD, NAD redox potential, RBC endothelial adhesiveness, hematologic parameters, frequency of painful crises, no. of hospitalization days, no. of painless days on study, and safety	Phase 2a, prospective randomized double-blind, placebo-controlled, crossover	L-glutamine 10 g/day or placebo; tid; Oral	24	Subjects with sickle cell anemia (homozygous Hb SS)	53 weeks	Completed; Summary Report
Efficacy and Safety	10478	5.3.5.1	To evaluate the effect of L-glutamine treatment on the occurrences of painful sickle cell crises, frequency of hospitalizations and emergency room visits, and safety	Phase 2, prospective, randomized, double-blind placebo-controlled, parallel-group	L-glutamine 0.3 g/kg/day or placebo; bid; Oral	L-glutamine: 37 placebo: 33	Subjects with sickle cell anemia or sickle β^0-thalassemia	51 weeks	Completed; CSR 10478
Efficacy and Safety	GLUSCC09-01	5.3.5.1	To evaluate the effect of L-glutamine treatment on the occurrence of sickle cell crises, frequency of hospitalizations and emergency room/medical facility visits, and safety	Phase 3, prospective, randomized, double-blind, placebo-controlled, parallel-group	L-glutamine 0.3 g/kg or placebo; bid; Oral	L-glutamine: 151 placebo: 78	Subjects with sickle cell anemia or sickle β^0-thalassemia	51 weeks	Completed; CSR GLUSCC09-01
PD, Efficacy and Safety	8822	5.3.5.2	To evaluate the effects of 3 different daily doses of oral L-glutamine on change from baseline in NADH, NADH/NAD_T redox potential, and hematological parameters	Open-label, uncontrolled	L-glutamine 10 g/day, 20 g/day and 30 g/day; Oral	10 g/day: 6 20 g/day: 4 30 g/day: 7	Subjects with sickle cell anemia (homozygous Hb SS)	4 weeks	Completed; Summary Report

Abbreviations: ^{14}C-L-Glutamine = carbon-14-labeled glutamine, homozygous Hb SS = homozygous hemoglobin SS type, K_m = Michaelis Menton Constant, NAD = nicotinamide adenine dinucleotide, NAD^+ = oxidized nicotinamide adenine dinucleotide, NADH = reduced nicotinamide adenine dinucleotide, NADH/NAD_T redox potential = ratio of NADH to total NAD, total NAD = NAD^+ + NADH, NA = not applicable, PD = pharmacodynamics, RBC = red blood cell, RTC = reticulocyte control, SCD = sickle cell disease, V_{max} = maximum velocity.

[a] Subjects did not receive test product. Blood samples were taken and treated in a lab setting with ^{14}C-labeled L-glutamine solution.

2.4.2. Pharmacokinetics

No studies have been performed by the Applicant to determine the pharmacokinetic characteristics of Xyndari. The application relies on scientific literature from 34 publications to describe the clinical pharmacology of glutamine as active substance.

The main PK parameters are derived from a study by Ziegler *et al.* 1990. In this study, six healthy male subjects were studied for orally administered glutamine, nine healthy volunteers (five male, four female) for a short-term glutamine infusion (constant IV infusion over 4 hours) and seven normal volunteers for a long-term glutamine infusion (glutamine added to parenteral nutrition), three isonitrogenous, isocaloric solutions by intravenous infusion for 5 consecutive days in studies separated by at least 2 weeks.

Absorption

After oral ingestion glutamine is rapidly absorbed from the lumen of the small intestine and transported to tissues for further utilisation. The uptake into cellular compartments is mediated by several membrane transporters (e.g., Bhutia & Ganapathy *et al.,* 2016; Pochini *et al.,* 2014) that regulate the homeostasis by coordinating absorption, reabsorption, and delivery to tissues. It is also known from literature that glutamine is not completely bioavailable after oral ingestion as a large percentage is captured by the splanchnic bed.

E.g., in Ziegler *et al.* 1990, absorption of glutamine has been investigated after single-dose oral exposure (0.1g/kg or 0.3g/kg) and after 4 hour i.v. infusion. For 0.1 g/kg glutamine the following results were obtained: C_{max}=1028 µM, T_{max}= 30 min, $t_{1/2}$= 106 ± 11 minutes and V_d= 512 ± 63 mL/kg. For 0.3 g/kg glutamine C_{max} was 1328 µM, T_{max}=45 min, $t_{1/2}$=117 ± 17 minutes and V_d=1254 ± 84 mL/kg. After an i.v. bolus the half-life was approximately 1 hour. The absorption of glutamine has not been discussed in terms of total systemic exposure (AUC). In Ziegler *et al* the 'integrated responses' (akin to AUC0-4hr) observed following 0 g/kg, 0.1 g/kg and 0.3 g/kg, were 4.4 ±5.2, 36.3 ± 6.8, 72.6 ± 12.7 µM.4h.103, demonstrating non-linearity.

Based on the presented information and the general knowledge about glutamine ADME, the absorption process and also the limited bioavailability after oral ingestion of glutamine as a substance are adequately defined *qualitatively*, however the quantification of respective PK parameters remains less certain, as there is relevant variability across cited studies, especially so for C_{max}. Although the intended treatment schedule foresees two oral doses per day in a chronic treatment setting, absorption and the systemic exposure after multiple oral dose administration have not been investigated.

The influence of food has not been studied directly but a proposal was made to recommend in section 4.2 of the SmPC to administer Xyndari together cold or room temperature beverage such as water, milk or apple juice, or 113 gram to 170 gram of food such as apple sauce or yogurt. No data on food interaction were presented in the evaluation, it is recommended that the impact of food on the exposure of glutamine should be evaluated in line with the EMA Guideline on the investigation of drug interaction (CPMP/EWP/560/95/Rev. 1 Corr. 2**) in a clinical study. The PK study data of glutamine should be presented as baseline corrected. In addition, this study may be conducted in patients with SCD, in order to enable characterising the PK of glutamine in the target population.

Distribution

After an intravenous bolus dose the volume of distribution was estimated to be approximately 200 mL/kg. The apparent volume of distribution (Vd) following i.v. administration of glutamine was similar to distribution within the extracellular fluid compartment (Vd: 210 ± 20 mL/kg). The Vd derived from the oral glutamine studies was 512 mL/kg [0.1g dose] and 1254 mL/kg [0.3 g dose] (Ziegler *et al.* 1990).

Metabolism

Endogenous glutamine undergoes extensive metabolism, and much of the ingested glutamine is rapidly catabolised within enterocytes. Intact, absorbed glutamine is catabolised in part by the liver. Key metabolic processes of glutamine involve hydrolysis to glutamate and ammonia via phosphate-dependent glutaminase. Through amidotransferase reactions, the amide nitrogen of glutamine serves as the precursor for the biosynthesis of many nitrogen-containing compounds. Glutamine is further involved in inter organ transport

of N, C and energy. Exogenous glutamine is anticipated to undergo similar metabolism. Metabolic clearance rates (MCR) of i.v. glutamine have been reported from different publications in healthy volunteers with rates of 2.8% per min (Darmaun *et al.* 1986), 558±48 mL/kg/h (Nurjhan et al. 1995), 620±20 mL/kg/h (Darmaun *et al.* 1991) and 496±57 mL/kg/h in subjects with short bowel syndrome (Darmaun *et al,* 1991).

Elimination

Elimination takes place mainly via metabolism with a relatively short half-life of a few hours; The half-life (t1/2) was reported for both oral and i.v. dosing (Ziegler *et al.* 1990). The t1/2 values after glutamine oral administration were 106 ± 11 minutes (0.1 g/kg dose) and 117 ± 17 minutes (0.3 g/kg dose). Following an i.v. bolus injection of glutamine solution, blood concentrations of glutamine declined in 2 phases, which is compatible with a 2-compartment elimination model. The t1/2 of the initial rapid distribution phase was 12 ± 2 minutes, followed by a terminal elimination phase with a t1/2 of 67 ± 11 minutes (Ziegler et al. 1990). Another study reported a t1/2 value of 25 minutes after a continuous i.v. infusion of glutamine (Darmaun *et al.* 1986).

Excretion only plays a minor role in glutamine clearance. Although glutamine can initially be eliminated by glomerular filtration, it is then almost completely reabsorbed by the renal tubules. After an intravenous bolus dose, the terminal half-life of L-glutamine was approximately 1 hour.

Pharmacokinetics of metabolites

The metabolic fate of glutamine is diverse with many metabolites, most commonly including CO_2, ammonia, alanine, lactate, citrulline, glutamate, aspartate, pyruvate and proline among others. Consequently, metabolites of glutamine are involved in a variety of biological processes. Pharmacologically particularly relevant metabolites include glutamate and ammonia, which are generated by hydrolysis of glutamine via glutaminase. Glutamate can further be metabolised to glutathione, proline, ornithine, and arginine or undergo catabolism to yield either CO2 or glucose (via hepatic and renal gluconeogenesis), with the nitrogen being excreted either as urea or ammonia.

Glutamate, ammonia and other amino acid levels were measured in Ziegler *et al.* 1990 following oral and IV glutamine administration. Oral administration of glutamine resulted in a significant increase in described metabolites. Glutamine and ammonia concentrations were not increased significantly. A schematic overview and discussion of the glutamine metabolism was provided. In healthy individuals levels of glutamate and ammonia concentrations were not increased significantly. In paediatric oncology patients dose levels of 0.75 g/kg reached ammonia levels not acceptable to neonatologists (155µM).

Consequences of possible genetic polymorphism

No CYP enzymes are involved in glutamine metabolism. A literature search performed by the Applicant did not retrieve any information about potential enzyme polymorphisms involved in glutamine metabolism. The Applicant has provided information to address potential higher glutamine transport rates in patients with SCD, although the literature is clearly sparse and conclusive data are not available.

Dose proportionality and time dependencies

Dose proportionality after single dose was assessed in Ziegler *et al.* 1990 where it was found that glutamine blood concentrations increased proportional to administered dose. Oral administration of 0.1 and 0.3g glutamine led to dose proportional blood concentrations: Cmax of 1028 µM (0.1g) or 1328 µM (0.3g) with

peaks between 30 to 45 minutes following ingestion, then declining over 1.5 to 2.0 hours post-dosing for the low dose (0.1 g/kg) and 3 to 4 hours post-dose for the high dose (0.3 g/kg). The integrated area described by the Gln concentration curve rose in a nonlinear fashion.

Systemic exposure after multiple oral dosing was not investigated. No PK profile after repeated administrations has been characterised.

Intra- and inter-individual variability

Factors potentially affecting Xyndari PK have neither been discussed theoretically nor explored in clinical studies with a view on intra- and inter-individual variability, particularly so for the concerned target population. Individual glutamine plasma levels were not measured for the phase II and phase III studies meaning that no statements could be made based on exploratory analyses on pivotal data either. Weight-based dosing has been used in phase 2/3 studies.

Pharmacokinetics in target population

In one of the presented publications (Niihara et al. 1997), V_{max} of glutamine transport kinetics was found substantially higher for sickle RBCs compared with those in normal control RBCs. At the same time, in a publication by Morris et al. 2010 the PK of 10g oral glutamine was investigated in five SCD patients, showing an increased plasma glutamine concentration of about 1000µM after ~30 min, which is by and large in line with the parameters for Cmax & tmax reported by Ziegler et al. 1990 in healthy males. However, the administered dose (10 mg) is different from those administered in the study Ziegler et al. (0 g/kg, 0.1 g/kg and 0.3 g/kg).

Special populations

Specific PK data exploring the relevance of important subgroups (i.e. renal/hepatic impairment, older patients, children, etc.) were not provided.

Pharmacokinetic interaction studies

No pharmacokinetics studies that examined the interaction between glutamine and drugs commonly used by patients with SCD, like hydroxyurea, analgesics, opioids, antibiotics and anticoagulants have been performed.

2.4.3. Pharmacodynamics

Information regarding the pharmacology of glutamine has been retrieved from publicly available scientific literature.

Mechanism of action

The Applicant presented information from 8 publications investigating the role of the pyridine nucleotide NAD and its reduced form NADH during oxidative damage in sickle RBCs. The studies found that the ratio of NADH to total NAD and therefore the NAD redox potential is decreased in sickle cells compared to normal RBCs.

This is explained by increased intracellular NAD^+ levels. Glutamine serves as a precursor for NAD and exogenous supply with glutamine might enhance NAD synthesis and NAD redox potential. Due to the assumed protective function of the NAD redox potential, RBCs may, as a result, be less affected by oxidative stress and damage. In the publication by Niihara et al., 2005, a reduced adherence of RBC from patients treated with L-glutamine to human umbilical vein endothelial cells in vitro was indicated, however these data are very limited.

Primary and Secondary pharmacology

The Applicant has provided evidence of the primary pharmacodynamic effect of glutamine in SDC in the form of literature and results from 2 legacy studies (a pilot study in 7 SCD patients (Study 8288 [Niihara *et al,* 1998]) and a dose finding study (8822)).

Results from study 8288 show an increase from 47.5±6.3 to 72.1±15.1nmol/ml in the mean NADH level at 4 weeks with a resulting increase of 47.2 ± 3.7 to 62.1 ± 11.8% in NAD redox potential (ratio of NADH to total nicotinamide adenine dinucleotide [NADT]). There was a trend upward in total NAD. Haemoglobin levels did not change significantly after 4 weeks of treatment with glutamine. Subjective clinical responses suggested an improvement over the 4 week treatment period.

No data concerning secondary pharmacology have been presented by the applicant.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

No studies have been performed by the Applicant to characterise the pharmacokinetic properties of oral glutamine at the intended dose levels and chronic administration scheme.

The cited literature reports used different formulations and administration routes, including oral, intravenous, nasogastric and parenteral nutrition across a broad range of dose levels. Even though none of the referred-to studies referred used the product intended for marketing, the data, limited as they are, are still deemed of relevance in principle. Glutamine is the active substance and potential differences in impurity profiles resulting from the production process (i.e., fermentation) are unlikely to impact the pharmacokinetics to an important degree.

Absorption, distribution, metabolism and elimination of glutamine are well known and sufficiently characterised qualitatively. However, although glutamine is an endogenous compound and the most abundant free amino acid in human muscle and plasma, the PK profile of Xyndari as chronically administered exogenous agent has not been conclusively established quantitatively. A dedicated PK study with the commercial product in SCD patients would have been useful; the lack of any PK sampling in the phase-II and phase-III studies is an additional shortcoming in this regard.

It was clarified that no additional PK data can be provided from the Xyndari phase-II/III studies. Exposure (i.e., Cmax & AUC) seem to increase with dose in the relevant dose range after single administrations, but there are only very limited data on the systemic exposure after multiple doses and potential changes of the PK profile over time.

Factors potentially affecting Xyndari PK and resulting subgroups of relevance have neither been discussed theoretically nor explored in clinical studies with a view on intra- and inter-individual variability. It is noted

however that weight-based dosing has been used in phase II/III. There may however be patient factors potentially influencing Xyndari PK that may be of relevance for a recommended posology and/or B/R assessment for respective subgroups of the target population. This particularly pertains to the possibility of an altered PK and/or accumulation of glutamine or glutamine metabolites and related risks in patients with impaired renal or hepatic function. Furthermore, no information on food interaction is available and conducting a respective PK study in the intended target population is suggested.

Taken together, the data presented so far do not enable conclusively describing the pharmacokinetic profile of Xyndari in SCD.

Pharmacodynamics

Information regarding the pharmacology of glutamine and its role in decreasing oxidative stress in RBCs, presumably leading to decreased sickling and/or reduced adherence of sickle RBCs to endothelial cells in patients with SCD has been retrieved from publicly available scientific literature and from the early stages of the clinical development programme. It is hypothesised that glutamine serves as a precursor for NAD and exogenous supply with glutamine might enhance NAD synthesis and NAD redox potential. Due to the assumed protective function of the NAD redox potential, RBCs may be less affected by oxidative stress and damage. The presented mode of action is in principle considered possible, but an impact on sickling, endothelial adhesion and vaso-occlusion is not firmly established empirically.

The pharmacodynamic parameters of interest that have been investigated/discussed by the Applicant include levels of NADH, total NAD and redox potential. Although glutamine-related decrease in adhesion of RBCs to endothelial cells was mentioned, the respective data are not available. Only results from the publication by Niihara et al., 2005, indicate a reduced adherence of RBC from patients treated with L-glutamine to human umbilical vein endothelial cells, however these data are very limited. No additional (*in-vitro*/*ex-vivo*) evidence supporting the proposed mode of action such as the glutamine effect on the morphology of erythrocytes (e.g., reduced sickling) and the reduced adhesive capacity of erythrocytes to the endothelium and clotting was provided.

Results from the 8288 pilot study (Niihara *et al.* 1998) and the 8775 dose-finding study indicate that NADH and the NAD redox potential increased after glutamine intake compared to baseline levels but these results lack clinical context. In the dose finding study (8822), no changes in NADH, NAD levels or redox potential were observed for 10g and 20g dosing groups. Even if it cannot be excluded that these doses were simply too low to exert an effect, this finding raises some concern regarding the validity of results for the 30g cohort and the importance of appropriate dosing and dose-related considerations for phase II/III.

No interaction studies have been performed by the Applicant. Patients with SCD are often treated with hydroxycarbamide, analgesics, opioids, antibiotics and anti-coagulants. The possible interactions with the frequently taken medication have not been sufficient addressed by the Applicant. Furthermore, no data concerning the relationship between plasma concentration and effect have been presented by the Applicant, nor has this issue been discussed theoretically.

The Applicant states that despite the limited understanding of the mechanism of action, the primary pathophysiology, polymerisation of haemoglobin S and increased adhesion of red blood cells can be extrapolated to other sickle genotypes.

Not measuring glutamine and relevant PD parameters besides the set of efficacy endpoints in phase-II/III remains an important shortcoming of the clinical development programme; no definite conclusions on the

relationship between exposure and effect can be drawn. NAD/NADH levels, morphological changes or adhesive capacity have not been measured during the phase II or III studies.

2.4.5. Conclusions on clinical pharmacology

Several uncertainties on Xyndari pharmacology remain. These are related to the applicability of literature PK data for quantitative statements on Xyndari PK in the intended target population including special populations.

The need for a food interaction/PK study has been identified. Characterisation of the PK in the target population in such a study would be of value.

Taken together, the presented data also do not conclusively establish the mechanism of action and the pharmacodynamic profile of glutamine in SCD.

2.5. Clinical efficacy

The primary efficacy data supporting this submission are based on 1 single pivotal long-term controlled Phase 3 trial (GLUSCC09-01) in patients with SCD, consisting of a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period. It evaluated the efficacy, safety, and tolerability of oral glutamine at doses of 0.3 g/kg of patient body weight, twice daily for 48 weeks, with an upper limit of 30 g/day, against placebo.

Supportive efficacy and safety data come from Study 10478, a Phase 2 randomised, double-blind, placebo-controlled, parallel-group, multicentre study, designed similarly to GLUSCC09-01.

Additionally, 5 exploratory legacy investigator-initiated studies have been conducted in academic research environment. Of these, Study 8288 and Study 8822 evaluated changes in both the NADH level and NAD redox potential with glutamine at a daily dose of 30 g/day.

An overview of the main and legacy studies is provided in the table below.

Table 3: Summary of clinical studies

Trial ID	Number of Centres	Type of Study	Study Design and Type of Control	Treatment Dose, Route Regimen	Duration of Treatment	No. of Patients Who Received Study Drug	Diagnosis of Patients
Study 8288 (Niihara et al, 1998)	1	Pilot study	Open-label, uncontrolled	Glutamine 10 g, Oral, tid	4 weeks	Glutamine: 7	Patients with sickle cell anaemia (homozygous S)
Study 8822	1	Dose-finding study	Open-label, uncontrolled	Glutamine 10 to 30 g, Oral	4 weeks	Glutamine: 11	Patients with sickle cell anaemia
Study 8775	1	Phase 2a safety and efficacy	Double-blind, placebo-controlled, crossover	Glutamine 10 g / Placebo OR Placebo / Glutamine 10 g, Oral, tid	53 weeks	Glutamine: 17 Placebo: 15[a]	Patients with sickle cell anaemia (homozygous S)
Study 10779	1	Exercise study	Open-label, controlled	Glutamine 30 g, Oral, bid	12 weeks	Glutamine: 14	Patients with sickle cell anaemia or control
Study 10511	1	Exercise tolerance study	Randomised, double-blind, placebo-controlled, parallel-group	Glutamine 0.3 g/kg or placebo, Oral, bid	12 weeks	Glutamine: 5 Placebo: 10	Patients with sickle cell anaemia or sickle β^0-thalassaemia
Study 10478	4[b]	Phase 2 safety and efficacy study	Randomised, double-blind, placebo-controlled, parallel-group	Glutamine 0.3 g/kg or placebo, Oral, bid	51 weeks	Glutamine: 36 Placebo: 33	Patients with sickle cell anaemia or sickle β^0-thalassaemia
Study GLUSCC09-01	31[c]	Phase 3 safety and efficacy study	Randomised, double-blind, placebo-controlled, parallel-group	Glutamine 0.3 g/kg or placebo, Oral, bid	51 weeks	Glutamine: 151 Placebo: 78	Patients with sickle cell anaemia or sickle β^0-thalassaemia

Abbreviations: bid = twice daily, ID = identification, tid = three times a day

[a] A total of 13 patients received both treatments, 2 patients received only placebo, and 4 patients received only glutamine.

[b] There were initially 5 sites; however, Site 106 was removed from the study for potential misconduct.

[c] One site (investigator: Hussein) was closed and another site (investigator: Woods) replaced it; both sites were considered Site 20.

2.5.1. Dose response studies

The Applicant has tested a dose of approximately 0.3 mg/Kg in the main Phase 3 and Phase 2 clinical studies. The dose selection was also based on review of literature for the use of glutamine in other conditions than in SCD. Supportive data on dose-response come from 2 of the legacy studies.

In **Study 8288** (pilot study), 7 subjects with diagnosis of sickle cell anaemia (homozygous HβS) received 10 grams of pure glutamine three times a day orally for 4 weeks. After overnight fasting, blood samples were drawn for analysis of total NAD, NADH, and NAD redox potential. Results showed an increase in NAD redox potential (ratio of NADH to NAD+ plus NADH, i.e., total NAD) after 4 weeks of Glutamine administration.

Study 8822 was a dose-finding study of Glutamine in patients with Sickle Cell Disease. It evaluated the effects of 3 different daily doses of oral Glutamine in 11 adult subjects with sickle cell disease (10 g/day (N = 6), 20 g/day (N = 4), or 30 g/day (N = 7) on change from baseline in NADH, NADH/NADT redox potential, and haematological parameters. Doses were administered in 2 or 3 evenly divided doses, for a total of 4 weeks. The Glutamine powder was mixed with a glass of water, juice, or soft drink for administration.

Whole blood extracts were assayed for NAD and NADH levels, using spectrophotometric enzymatic cycling assays, at baseline. Additionally, assays were run at the 2-week and 4-week time point following treatment with Glutamine. Some subjects participated in more than one dose group after a washout period. Only in the 30 g/day dose group, there was a consistent increase in mean NADH and NAD redox potential (see table below).

Table 4: Results of the Dosing at 20 g/day Versus 30 g/day of Oral Glutamine for 4 Weeks (Mean ± 1SD).

	20 g/day L-glutamine (N = 4)		30 g/day L-glutamine (N = 7)	
	Baseline (Week 0)	Week 4	Baseline (Week 0)	Week 4
NADH (nmol/ml RBC)	51.6 ± 6.8	45.6 ± 8.6	47.5 ± 6.3	72.1 ± 15.1
NAD_T (nmol/ml RBC)	100.8 ± 6.4	102.5 ± 21.2	101.2 ± 16.0	116.4 ± 14.7
$NADH/NAD_T$ (%)	51.4 ± 8.4	46.4 ± 15.8	47.2 ± 3.7	62.1 ± 11.8

Based on these data, the Applicant selected the dose of 0.3 mg/Kg, with a maximum of 30 g/day. No dose-or blood-level responses were analysed in either Study 10478 or Study GLUSCC09-01.

2.5.2. Main study

Study GLUSCC09-01: A Phase III, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study of L-Glutamine Therapy for Sickle Cell Anemia and Sickle ß0-Thalassemia.

This was a phase 3, prospective, randomised, double-blind, placebo-controlled, parallel-group, multicentre study to evaluate the safety and efficacy of oral glutamine therapy for patients with sickle cell anaemia or sickle ß0-thalassemia who were at least five years old. The study consisted of a 4-week screening period, a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period for a total duration of up to 57 weeks.

Methods

Study Participants

The patient population consisted of individuals with sickle cell anemia (homozygous SS) and sickle ß0-thalassemia. Up to 225 patients were planned to be randomized at up to 35 sites in the United States.

Inclusion Criteria

- Patient is at least 5 years of age.
- Patient has been diagnosed with sickle cell anaemia or sickle ß°-thalassemia (documented by haemoglobin electrophoresis).
- Patient has had at least two documented episodes of sickle cell crises within 12 months of the screening visit.
- If the patient has been treated with an anti-sickling agent within three months of the screening visit, the therapy must have been continuous for at least three months with the intent to continue for the duration of the study.
- Patient or the patient's legally authorised representative has given written informed consent.

- If the patient is a female of child-bearing potential, she agrees to avoid pregnancy during the study and is willing and agrees to practice a recognised form of birth control during the course of the study (e.g. barrier, birth control pills, abstinence).

Exclusion Criteria

- Patient has a significant medical condition that required hospitalisation (other than sickle cell crisis) within two months of the screening visit.
- Patient has prothrombin time INR > 2.0.
- Patient has serum albumin < 3.0 g/dl.
- Patient has received any blood products within three weeks of the Screening Visit.
- Patient has uncontrolled liver disease or renal insufficiency.
- Patient is pregnant or lactating or has the intention of becoming pregnant during the study (if female and of child-bearing potential).
- Patient is currently taking or has been treated with any form of glutamine supplement within 30 days of the screening visit.
- Patient has been treated with an experimental anti-sickling medication/treatment within 30 days of the screening visit (with the exception of hydroxyurea in pediatric patients).
- Patient is currently taking or has been treated with an investigational drug within 30 days of the screening visit (with the exception of hydroxyurea in pediatric patients).
- Patient is currently enrolled in an investigational drug or device study and/or has participated in such a study within 30 days of the screening visit.
- There are factors that would, in the judgment of the investigator, make it difficult for the patient to comply with the requirements of the study.

Treatments

An equivalent volume of oral powder, glutamine or placebo, was administered at a dosage of 0.3 g/kg of patient body weight, twice daily (bid) for 48 weeks. Patients were instructed to mix the powder immediately before ingestion with water or other non-heated beverage other than alcohol or with any non-heated food (eg, yogurt, applesauce, or cereal). Mixing glutamine with soda or highly acidic juices (such as grapefruit juice or lemonade) was not recommended. The dosage was increased by increments of 10 g/day based on body weight. The upper limit of the total daily dose was 30 g for patients. After 48 weeks of treatment, the dose was tapered to zero over 3 weeks. Patients were weighed at each study visit and if a patient's weight change was maintained over 2 consecutive study visits, the study drug dosage was adjusted accordingly.

The approved anti-sickling agent, hydroxycarbamide was permitted. Therapy with hydroxycarbamide was to have been continuous for at least 3 months prior to screening and there must have been an intent to continue the therapy for the duration of the study. All concomitant medications were recorded throughout the course of the study.

Objectives

Primary objective:

- To evaluate the efficacy of oral glutamine as a therapy for sickle cell anaemia and sickle ß0-thalassemia as evaluated by the number of occurrences of sickle cell crises.

Secondary objectives:

- Assessment of the effect of oral glutamine on:
  a) frequency of hospitalisations for sickle cell pain;
  b) frequency of emergency room/medical facility visits for sickle cell pain;
  c) haematological parameters (haemoglobin, haematocrit, and reticulocyte count);
- Assessment of the safety of glutamine as a therapy for sickle cell anaemia as evaluated by adverse events, laboratory parameters, and vital signs.

Outcomes/endpoints

Primary efficacy endpoint: the number of sickle cell crises through Week 48 and prior to start of taper.

Secondary efficacy endpoints: number of sickle cell crises through Week 24; number of hospitalisations for sickle cell pain through Weeks 24 and 48; number of emergency room/medical facility visits for sickle cell pain through Weeks 24 and 48; and haematological parameters (haemoglobin, haematocrit, and reticulocyte count).

- Other efficacy endpoints: height, weight, and growth curve.

- Safety endpoints: incidence of adverse events (AEs), safety laboratory results, and vital signs.

An independent central adjudication committee (CAC) was used to evaluate whether reported SCCs, as well as hospitalisations and emergency room (ER)/medical visits related to SCCs, met the criteria of the efficacy outcome. The committee was composed of 3 haematology/oncology physicians.

Events determined to be SCCs by the CAC were included in the efficacy analyses if they met any 1 of the following relevant predefined criteria:

- A visit to an ER or medical facility for sickle cell disease-related pain treated with a parentally-administered narcotic or ketorolac: If facility policy clearly documented the non-use of parental narcotic or parenteral ketorolac, a visit to an ER or medical facility for sickle cell disease-related pain that resulted in administration of oral narcotic, oral ketorolac, or other oral non-narcotic pain relievers was also adjudicated as an SCC.

- Acute chest syndrome (ACS): defined as 1 or more acute clinical pulmonary findings corroborated by findings of a new pulmonary infiltrate on chest x-ray films. Clinical pulmonary findings included the following: fever, cough, sputum production, dyspnoea, or hypoxia. ACS was considered to be an SCC even if the symptoms were not painful enough to require narcotics or ketorolac.

- Priapism: considered to be an SCC even if the symptoms were not painful enough to require narcotics or ketorolac.

- Splenic sequestration: defined as an increase in spleen size associated with localised pain along with a greater than or equal to 2 g/dL decrease in haemoglobin concentration within 24 hours. Splenic sequestration was considered to be an SCC even if the symptoms were not painful enough to require narcotics or ketorolac.

Sample size

The sample size required for the study was calculated to be 220, with 147 patients assigned to glutamine therapy and 73 patients assigned to placebo.

The study was expected to have a 25% dropout rate, with an estimated 110 patients on glutamine completing the study and 55 patients on placebo completing the study; the number of completed subjects in each treatment group provides 80% power to detect a difference between the groups in the distribution of the number of sickle-cell crises at Week 48. Randomization was stratified by both investigational site and hydroxycarbamide use. Sample size calculation was based on a significance level of 0.048, and power was calculated based on the Wilcoxon rank-sum test for ordered categories. The power was based on testing the null hypothesis of no difference in the probability distribution of the number of sickle cell crises at Week 48 versus the alternative hypothesis that the probability distribution is different between the two groups.

Randomisation

Patients were randomized in a 2:1 ratio of glutamine or placebo and stratified by investigational site and hydroxycarbamide usage.

Blinding (masking)

The investigators, site staff, patients, and study personnel were blinded to treatment assignment. Both glutamine and placebo were white powders with an identical appearance. The placebo was equivalent in volume to the study medication. In addition, to ensure the blinding of the taste, the powders were mixed immediately before ingestion with a beverage or food for administration. For the interim analysis, the treatment blind was maintained for the final analysis by (a) having an independent statistician perform the analysis, and (b) using a series of protocol specific procedures (PSPs) to ensure maintenance of the blind.

Statistical methods

Analysis data sets

The primary efficacy analysis was planned to be based on the ITT population, defined as all randomized subjects. The definition of the per-protocol set (all patients who were enrolled in the study for at least 12 weeks, and who took at least 50% of the study medication over the course of their participation in the treatment period without significant protocol violations) and the safety analysis set (all patients who received at least one dose of study medication) are also acceptable, but an unambiguous definition of what constitutes a "significant protocol violation" was not pre-specified.

Statistical methods for efficacy analysis

A single interim analysis was planned to be performed when 80 patients have completed 24 weeks of the study. Parameters to be analyzed included all secondary efficacy parameters excluding parameters measured at the 48-week time point. Significance levels were adjusted to accommodate the single interim analysis and preserve the overall type-I error of 0.05. A flexible fixed-sequence testing method was used (Huque, 2008), with the single interim analysis performed at the 0.005 significance level. The significance level for the final analysis depended on the acceptance or rejection of the null hypothesis of the interim analysis. Specifically, if the null hypothesis of the interim is rejected the null hypothesis of the second (final analysis) was planned to be performed at the 0.05 significance level, however, if the null hypothesis of the interim is not rejected the null hypothesis of the final analysis was planned to be performed at the 0.045 (α=0.05-0.005) significance level.

All statistical tests of the interim were performed against a two-sided alternative hypothesis, and declared statistically significant when the calculated p-value is $\leq$ 0.005. In the final analysis only the primary endpoint, the number of painful sickle cell crises at week 48, was adjusted as described above. All secondary endpoints in the final analysis were considered supportive of the primary endpoint and were tested at the 0.05 significance level.

Multiplicity adjustment accounting for multiple/sequential testing is acceptable. However, the ultimate goal of the interim analysis remains unclear; different endpoints and analysis data sets were considered in the interim and the final analysis. While the final analysis was planned to be based on the comparison of the primary endpoint (at week 48) in all randomized patients, the interim analysis was based on week 24 efficacy endpoints in patients who completed at least 24 weeks on study medication, took at least 50% of the study medication during that time without significant protocol violations (and there was no pre-specification of what constitutes a major protocol violation, as mentioned above). The null hypothesis of the interim analysis could not be rejected in the interim analysis at the 0.005 significance level, and the trial continued. As a consequence, the primary endpoint in the final analysis needs to be tested at the significance level of 0.045, and the confidence intervals need to be adjusted accordingly, such that coverage of at least 95% is assured. Measures to limit the knowledge of the interim results to a small group of independent experts and to maintain trial integrity appear appropriate. The Applicant clarified that the interim results, which were based on 80 patients, who completed 24 weeks of treatment, indicated a favourable outcome in the glutamine group with respect to the efficacy endpoints. The interim analysis report was not provided for assessment. The interpretation of the treatment effect estimates of the primary efficacy analysis is based on a confidence level that accounts for the interim analysis.

The treatment groups were compared with respect to number of painful sickle cell crises (the primary endpoint) using a Cochran-Mantel-Haenszel (CMH) test (row mean scores) with ranks as scores and controlling for investigational site and hydroxyurea use at baseline. The number of painful sickle cell crises and the number of hospitalizations for sickle cell pain were analysed in the same way. It was argued that the CMH test using 'modified ridit scores' actually represented the 'original pre-specified analysis'. However, the specification of the statistical methods in the analysis plan (before breaking the blind) should not leave any room for ambiguity and it is not agreed that the originally used method SCORES=RANK does not account for stratification. It is rather the impact of the strata that is weighted differently.

The CMH approach does not provide estimates of the treatment differences that can easily be interpreted and does not adequately take the observation period of the subjects into consideration, and there are considerable differences in study/drug discontinuation between treatment arms. A statistical analysis that takes different observation periods of subjects into account and that enables the quantification of the treatment effect by appropriate interval estimates (e.g. confidence intervals of the sickle cell count rate ratio

that account for the group sequential testing and have a coverage of at least 95%) was provided, i.e. analyses using a negative binomial regression (NBR) including treatment, region and HU use as covariates, and log (time on study) as an offset (please refer to issues raised on the handling of missing data).

The primary efficacy evaluation has to address all relevant post-randomisation (intercurrent) events that may affect the interpretation of the treatment difference. Treatment discontinuation is considered a relevant intercurrent event, and the treatment effect to be quantified (estimand) needs to be defined with respect to treatment discontinuation. Post-withdrawal data were not collected, however. Thus, plausible and sufficiently conservative (i.e. not favoring experimental treatment) assumptions are made about the outcome of these withdrawing patients.

It is not justifiable that the SCC intensity observed until treatment discontinuation remains unchanged in the remaining period. Another event that may impact the outcome variable is the change in hydroxyurea treatment/dose in the course of the study. The Company however clarified that HU use was stable in both study arms. Thus, it is concluded that the change of HU use does not represent an important event impacting efficacy comparisons.

Missing values were planned to be replaced in the following way: For patients who discontinued prior to week 48, painful sickle cell crises count was imputed using the mean number of crises for the patients of the same treatment group who did complete week 48. If the imputed count was less than the crises count at the time of discontinuation, the latter was used. This approach was criticized as it may overestimate the treatment effect. Therefore 4 different missing data imputation schemes were presented:

- Imputation Scheme #1 assumes a worse outcome for patients who withdraw than those who complete from the same treatment group. Sickle cell crisis (SCC) count for withdrawals will be the largest number of SCCs observed for completers in the same treatment group.
- Imputation Scheme #2 assumes a worse outcome for patients who withdraw than those who complete. SCC count for withdrawals will be the largest number of SCCs observed for all completers.
- Imputation Scheme #3 is based on the placebo group. The SCC count for all withdrawals will be the mean number of SCCs for placebo completers, or actual SCC count (whichever is higher).
- Imputation Scheme #4 assigns the SCC count for all withdrawals as the mean number of SCCs for completers overall (i.e. not by treatment group), or actual SCC count (whichever is higher).

Scheme 3 is considered the most appropriate one. However, as for the other schemes, the approach does not differentiate between reasons for discontinuation. Some of the reasons may be indicative of worsening of the disease course, and thus, the treatment effect may be overestimated using this placebo-based imputation technique. On the other hand, it appears difficult to make sensible assumptions on the extent of worsening due to the limited documentation upon withdrawal. Results from sensitivity analyses using multiple imputations based on placebo patients were subsequently provided (see results section below).

In conclusion, imputation scheme 3 (placebo imputation) using negative binomial regression, (employing multiple imputation) is considered an appropriate approach to address missing observational periods following study withdrawal, but may still slightly overestimate the treatment effect.

Safety Analysis

Safety analyses were performed on the safety population with no imputation of missing values. Safety endpoints included incidence of adverse events, safety laboratory results, and vital signs (see section 4 for details).

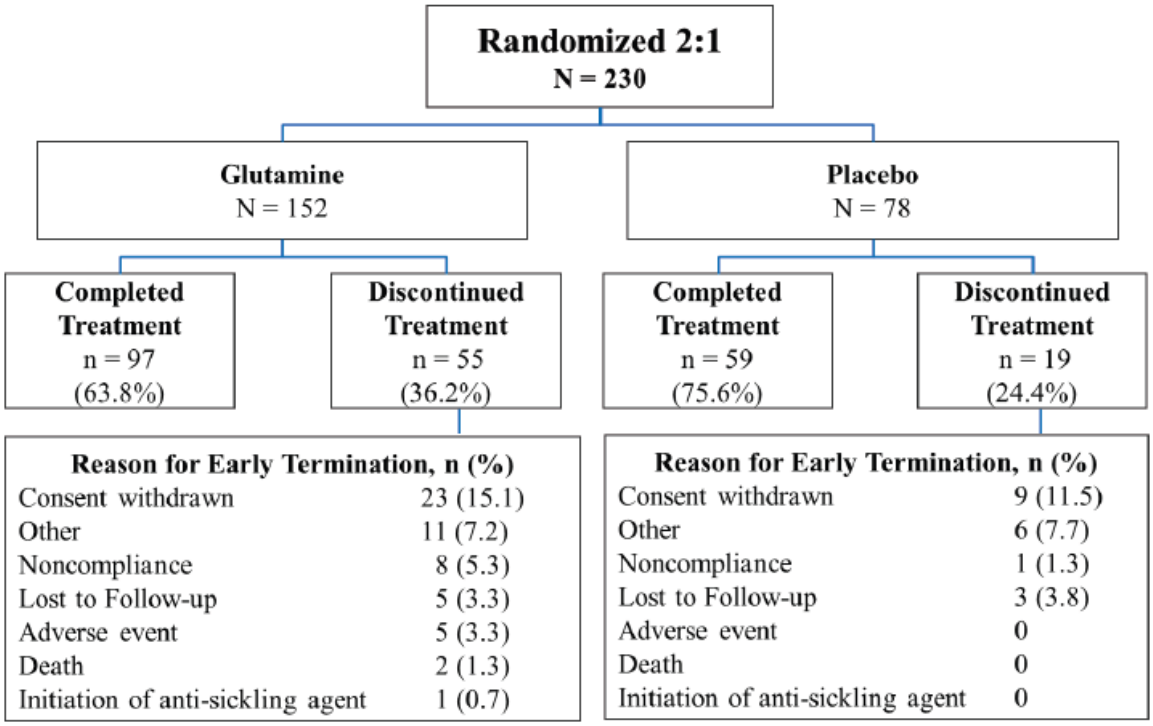
Results

Participant flow

A total of 230 patients were randomised in the study (intent-to-treat population). Of these, 152 patients were in the glutamine group and 78 were in the placebo group. One patient in the glutamine group did not receive any dose of the medication and was removed from analysis that included time in studying the model.

A total of 156 patients completed the study, 63.8% (97/152) in the glutamine group and 75.6% (59/78) in the placebo group. The most frequent reason for early termination was "Consent withdrawn" in both groups.

Figure 3: Patient Disposition - Study GLUSCC09-01 (ISE ITT Population).



Recruitment

This was a multicenter study, with patients being enrolled at 31 active sites in the USA. The first patient was enrolled on 21 June 2010 and the last patient completed on 19 December 2013. The study lasted 3 years and 6 months.

Conduct of the study

The original protocol (dated 19 October 2009) was amended five times. The amendment dates and key changes for each amended protocol are listed and described below.

Table 1 Protocol Amendments

Original Version Date	Amendment Version Date	Key Changes
19 October 2009	28 January 2010	• Made change in exclusion criterion • Updated definition of a sickle cell crisis • Corrected sample size calculation from 207 to 220 patients • Clarified that non-compliant patients will not be withdrawn from the study • Added section on additional efficacy analyses • Clarified secondary efficacy endpoint definitions • Added a new section on Central Adjudication Committee
28 January 2010	18 March 2010	• Updated inclusion and exclusion criteria • Clarified that serum pregnancy test will be performed for all females at Screening Visit, not just those classified as child bearing potential • Clarified which foods/beverages could be mixed with investigational product • Made change in the definition of primary efficacy endpoint to remove "painful" from the description of sickle cell crises • Added step in emergency unblinding procedure • Provided additional guidance regarding administration of investigational product • Updated supply of investigational product from 'four' to 'five weeks' • Clarified that all sickle cell disease related events for which patients seek medical attention will be reported as AEs • Updated sample size for both test and placebo groups • Clarified how safety would be monitored during study • Clarified who can perform physical examinations
18 March 2010	16 February 2011	• Updated exclusion criteria • Increased number of potential clinical sites • Clarified that screening complete blood count (CBC) and reticulocyte count laboratory values can be used as baseline laboratory, as long as the Screening Visit laboratory was within 1 week of Baseline Visit. • Updated reasons for patient withdrawal • Clarified SAE reporting procedure
16 February 2011	13 September 2011	• Revised definition of a sickle cell crisis
13 September 2011	26 March 2012	• Increased number of clinical sites from 30 to 35 • Revised definition of a sickle cell crisis

Baseline data

Demographics and Baseline Characteristics of the ITT Population are described in the following tables:

Table 5: Study GLUSCC09-01. Demographics and Baseline Characteristics (ITT Population)

	L-glutamine (N=152)	Placebo (N=78)	Total (N=230)
Age (yrs)			
n	152	78	230
Mean (SD)	22.4 (12.32)	21.4 (12.42)	22.0 (12.33)
Median	19.0	17.0	18.0
Min-Max	5-57	5-58	5-58
Age group n(%)			
5 - 7	12 (7.9)	6 (7.7)	18 (7.8)
8 - 12	22 (14.5)	11 (14.1)	33 (14.3)
13 - 18	41 (27.0)	26 (33.3)	67 (29.1)
19 - 30	38 (25.0)	18 (23.1)	56 (24.3)
31 - 65	39 (25.7)	17 (21.8)	56 (24.3)
Sex n(%)			
Male	73 (48.0)	33 (42.3)	106 (46.1)
Female	79 (52.0)	45 (57.7)	124 (53.9)
Race n(%)			
Black	144 (94.7)	73 (93.6)	217 (94.3)
Hispanic	4 (2.6)	3 (3.8)	7 (3.0)
Other	4 (2.6)	2 (2.6)	6 (2.6)

Table 6: Study GLUSCC09-01. Disease and Treatment History (ITT Population)

	All Randomised[a]	
	Glutamine N = 152	Placebo N = 78
SCCs in the year prior to screening		
Mean (SD)	3.9 (2.7)	4.1 (2.8)
Prior treatment with hydroxycarbamide		
Yes, n (%)	101 (66.4)	52 (66.7)
No, n (%)	51 (33.6)	26 (33.3)
If Yes,		
Time since first treatment, n	84	45
Mean (SD), years	4.4 (4.03)	4.4 (3.09)
Other experimental anti-sickling medication in the year prior to screening		
Yes, n (%)	0	2 (2.6)
No, n (%)	152 (100.0)	76 (97.4)
Diagnosis, n (%)		
Sickle cell anaemia	136 (89.5)	71 (91.0)
Sickle β^0-thalassaemia	14 (9.2)	7 (9.0)
Sickle β^+-thalassaemia	2 (1.3)	0

ITT = intent-to-treat, SCCs = sickle cell crises, SD = standard deviation.
[a] Primary efficacy population in Study GLUSCC09-01 (ITT).

Numbers analysed

As described in the SAP, all efficacy analyses were performed on the intent-to-treat population (230 patients); selected efficacy analyses were also performed on the per-protocol population (169 patients).

Also as described in the SAP, additional subgroup analyses were performed, including analysis by (a) region, (b) prior hydroxyurea use, (c) age, and (d) sex.

There were early withdrawals of 48 patients in the glutamine group and 15 patients in the placebo group. Three patients in the glutamine group and two patients in the placebo group were excluded from analysis due to: their diagnosis; not having at least 2 episodes of crises within 12 months of the Screening Visit; or not meeting other inclusion criteria.

Outcomes and estimation

Efficacy results

With the initial submission, the Applicant provided a pre-specified analysis that failed to reach statistical significance. Furthermore, post-hoc analyses showing significant results were shown.

Notwithstanding the Applicant´s argumentation on the original analysis plan not having been followed erroneously in the SAP (see further above), the statistical method as well as the missing data imputation were criticized.

Consequently, the Applicant provided additionally requested analyses, using NBR and 4 different schemes to impute missing data as outlined above. Those analyses (and specifically imputation scheme 3 - placebo imputation) are considered the most appropriate to inform on the benefits of glutamine.

The efficacy results shown below are structured in the following way to also reflect the assessment history (a similar structure is applied later on for presenting phase 2 study results):

(i) Newly requested analyses (using NBR for imputation scheme 3);

(ii) Pre-specified analyses (using a CMH test with ranks as scores);

(iii) Post-hoc analyses (using a CMH test with modified ridit scores).

i) Newly requested analyses using CMH and NBR analyses

Primary (Number of Sickle Cell Crises) and secondary efficacy analyses

Under Imputation Scheme #3, the SCC count for all withdrawals was replaced by the mean number of SCCs for placebo completers, or actual SCC count (whichever is higher).

The distribution table with this imputation is Table 36 and results of CMH and NBR testing is given in Table 37.

In these results the rate ratio favours glutamine (0.89), but neither the CMH (p = 0.2131) nor the NBR (p = 0.193) results were statistically significant.

Table 36. SCCs with Imputation Scheme #3 – Study GLUSCC09-01

Descriptive Statistics	Placebo (N = 78)	Glutamine (N = 152)
Mean (SD)	3.9 (2.54)	3.5 (2.27)
Median	4.0	4.0
Min, Max	0, 15	0, 15
Number of crises (n)		
0	4	15
1	10	16
2	11	17
3	4	13
4	23	65
5	12	8
6	5	6
7	4	5
8	2	2
9	1	3
11	1	1
15	1	1

max = maximum, min = minimum, N = total number of patients, n = number of patients, SCC = sickle cell crisis, SD = standard deviation

Source: Table Q100a.3

Table 37. CMH and NBR Analyses of Number of SCCs at Week 48 Using Imputation Scheme #3 – GLUSCC09-01

	Placebo (N = 78)	Glutamine (N = 152)
CMH test, controlling for region and HU use		
p-value	0.2131	
NBR[a] Modeling Results		
Rate Per 48 Weeks (95% CI)	3.97 (3.44, 4.59)	3.54 (3.16, 3.96)
Rate Ratio[b] (95.5% CI)	0.89 (0.75, 1.06)	
p-value[c]	0.193	

Imputation Scheme #3 is based on the placebo group. The SCC count for all withdrawals will be the mean number of SCCs for placebo completers, or actual SCC count (whichever is higher).
CI = confidence interval, CMH = Cochran–Mantel–Haenszel test, H_0 = null hypothesis, HU = hydroxycarbamide, NBR = negative binomial regression, SCC = sickle cell crisis.
[a] NBR model with Treatment, Region, and HU Use as main effects. One subject who was randomised but never took study medication was not included in the analysis.
[b] Rate ratio is (rate per 48 weeks for Glutamine)/(rate per 48 weeks for Placebo). A rate ratio < 1 favours Glutamine.
[c] p-value for H_0: No Difference Between Treatments.
Source: Table Q100a.3 and Table Q100a.7

The differences in SCC between glutamine and placebo were (point estimates): -0.9 in imputation scheme #1 (worst case scenario), 0.4 in imputation scheme #3 (placebo imputation) and 0.5 in imputation scheme #4 (imputation based on completers irrespectively of study arm). Of note, all of the 4 imputation schemes results were far from reaching statistical significance.

The estimated difference in the number of SCC compared to placebo varies greatly depending on the analysis and imputation methods used, being consistently small in all the imputation schemes presented by the Applicant. In the most realistic imputation scenario (scheme #3), the difference in the mean number of SCC was 0.4 with a rate ratio of 0.89 (0.75, 1.06).

Rate of hospitalisations and rate of emergency room (ER) visits per 48 weeks both show a NBR rate ratio consistent with the primary endpoint and in favour of glutamine (0.81 (95%CI: 0.61, 1.07)) and 0.73 (95%CI: 0.49, 1.08), respectively), although these comparisons were not statistically significant.

Additionally, requested analyses using NBR and multiple imputation based on placebo completers were submitted. For these results, imputation was only based on placebo *completers*, rather than accounting for all placebo subjects, which may have led to an overestimation of results.

Primary endpoint:

A rate ratio for SCCs favouring glutamine after 48 weeks of treatment (point estimate 0.82, 95.5%CI: 0.65, 1.04, p = 0.092) was calculated, which is not statistically significant. The respective mean values for SCCs are 3.54 vs. 3.97 for glutamine vs. placebo.

Secondary endpoint:

For analyses using multiple imputation and negative binomial regression, rate of hospitalisations and rate of emergency room (ER) visits per 48 weeks both showed a rate ratio consistent with the primary endpoint and

in favour of glutamine (0.82 (95%CI: 0.62, 1.08)) and 0.75 (95%CI: 0.51, 1.10), respectively), neither of which statistically significant.

Additional endpoints:

The rate ratio for acute chest syndrome (ACS) favoured L-glutamine (0.41, 95%CI: (0.18, 0.92)).

Cumulative duration of SCCs favoured the glutamine arm (rate ratio of 0.88, 95%CI: (0.63, 1.24)).

ii) Pre-specified analyses using a CMH test

Primary efficacy analysis

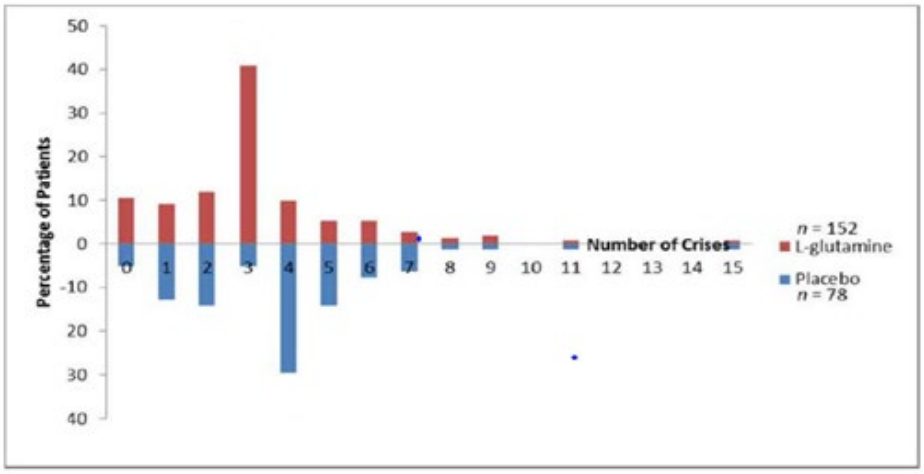
The pre-specified, primary efficacy analysis failed. In the ITT population, the median number of sickle cell crises through *Week 48* was 3 in the L-glutamine group compared to 4 in the placebo group, which was not a statistically significant difference (p=0.063). The median number of sickle cell crises through *Week 24* was 2 in the L-glutamine group and 2 in the placebo group (p=0.169). For the PP population, the results were similar.

Table 7: Number of SCCs –Intent-to-Treat population.

Interval	Treatment Group L-glutamine (N=152)	Placebo (N=78)	P Value
Through Week 48			
Mean (SD)	3.2 (2.25)	3.9 (2.53)	
Median	3	4	0.063
Range	0 – 15	0 – 15	
Through Week 24			
Mean (SD)	1.7 (1.58)	2.1 (1.69)	
Median	2	2	0.169
Range	0 – 8	0 – 7	

Source: Tables 14.2.2a and 14.2.2b
SD = standard deviation; Range = min - max
All p-values are from the Cochran-Mantel-Haenszel test controlling for stratification variables geographic region and hydroxyurea use.

Figure 4: Histograms of Number of Sickle Cell Crises in the Glutamine and Placebo Groups Through Week 48 (Intent-to-Treat Population).



Besides the fact that the primary analysis failed, there are a number of uncertainties with regard to the statistical analysis, as already stated above.

The Company also reported separate analyses for the comparative incidences of the different sub-events of the primary endpoint. Analyses excluding ACS, priapism and splenic sequestration from the primary analysis show consistency in the treatment effect. Nearly all patients with SCC had one or more event defined by the first component, i.e., a visit to an emergency room/medical facility for SCD-related pain, treated with a parental narcotic or toradol or an oral narcotic.

Secondary Efficacy Endpoints: Number of Hospitalizations and Emergency Room Visits for Sickle Cell Pain

In the ITT population, there were statistically significantly fewer *hospitalizations for sickle cell pain* through *Week 48* in the L-glutamine group than in the placebo group, with medians of 2 versus 3, respectively (p=0.041; Table 10).

The median numbers of *emergency room visits* in the L-glutamine group and the placebo groups for sickle cell pain through *Week 48* were essentially the same in both the ITT and PP populations.

Table 8: Number of Hospitalizations and Emergency Room vistis for Sickle Cell Pain (ITT population)

Variable and Interval	Treatment Group L-glutamine (N=152)	Placebo (N=78)	P Value
Number of hospitalizations for sickle cell pain			
Through Week 48			
Mean (SD)	2.3 (1.99)	3.0 (2.31)	
Median	2	3	0.041
Range	0 – 14	0 – 13	
Through Week 24			
Mean (SD)	1.2 (1.27)	1.6 (1.47)	
Median	1.0	2.0	0.070
Range	0 – 6	0 – 6	
Number of emergency room visits for sickle cell pain			
Through Week 48			
Mean (SD)	1.1 (1.49)	1.6 (2.30)	
Median	1	1	0.128
Range	0 – 12	0 – 15	
Through Week 24			
Mean (SD)	0.8 (1.26)	0.8 (1.23)	
Median	0	0	0.833

Range	0 – 10	0 – 7

Source: Tables 14.2.3a and 14.2.4a.
SD = standard deviation; Range = min - max
Note: All p-values are from the Cochran-Mantel-Haenszel test of treatment controlling for region and hydroxyurea use.

Additional secondary analyses (unplanned)

Three additional analyses were performed to evaluate the relevance of the primary and secondary efficacy findings: (1) ACS; (2) Analysis of time to first sickle cell crisis; and (3) Analysis of cumulative days in hospital. These analyses were performed without pre-specification in the SAP and therefore results are interpreted with caution.

Ad (1): *ACS* was found to be significantly improved with L-glutamine treatment with 26.9% of placebo treated patients compared to 11.9% of patients in the L-glutamine group experiencing ACS, (P=0.006, Fisher's Exact test). Analysis on time to first occurrence of ACS was performed using the Kaplan-Meier method.

In the post-hoc analysis, statistically significantly fewer (p = 0.0028) occurrences of ACS occurred in the glutamine group relative to placebo. Mean and median values were 0.1 vs. 0.3 and 0 vs. 0 in both groups, respectively. It is noted that no baseline data are available. Therefore, the results are interpreted with caution. ACS represents a serious complication and is one of the major reasons for hospitalization and a major cause of mortality. In this context, it would be considered of clinical benefit if the occurrence of such ACS episodes can indeed be reduced.

Ad (2): The median number of days before the first crisis was 87 days in the L-glutamine group compared to 54 days in the placebo group, a difference that was statistically significant (p=0.009) .

The post-hoc analysis provided with the 'new' database showed *a time to second SCC* (measured from the beginning of the study) of 212 days in the glutamine group vs 133 days in the placebo group. Furthermore, the *recurrent crisis event time* indicated that the average cumulative SCC count in the glutamine group was reduced by 25% compared to the placebo group over the entire 48-week treatment period. It is agreed that these results indicate that glutamine provides a protective effect over a longer time period; nevertheless, time-to-event analyses strongly correlate with SCC rates and provide thus only marginal additional insight into the treatment effect of glutamine.

Ad (3): The median number of days in hospital was statistically significantly shorter in the L-glutamine group (6.5 days) compared to 11 days in the placebo group (p=0.022) (Table 9).

Secondary Efficacy Endpoint: Hematological Parameters

There were no significant differences in the hematological parameters (hematocrit, hemoglobin, reticulocytes) at Week 48 or Week 24 between the L-glutamine group and the placebo group in the intent-to-treat population.

Other Endpoints: Height, Weight, and Growth Curve

Overall, there were no statistically significant between group differences in weight, height and growth curve change from baseline in most of the patient populations (categories: patients age 20 years or older and patients less than 20 years of age).

Safety outcomes related to efficacy

Some other interesting endpoints/parameters, as e.g. pyrexia or infections, were reported as safety events. They are considered related to efficacy and therefore presented here.

'Sickle cell anaemia with crisis' was the most commonly reported *TEAEs*: 152 patients [81.3%] in the glutamine treatment group and 97 patients [87.4%] in the placebo treatment group. Of these, 62 patients (33.2%) and 47 patients (42.3%) in the glutamine and placebo groups, respectively, had TEAEs of sickle cell anaemia with crisis that were considered *severe*. Sickle cell anaemia with crisis TEAEs were considered to be *related to study drug* in 2 patients (1.1%) in the glutamine group and 1 patient (0.9%) in the placebo group.

Other TEAEs potentially related to SCD:

- Pyrexia: 32 patients [17.1%] in the glutamine treatment group and 31 patients [27.9%] in the placebo treatment group.
- Upper respiratory tract infection: 25 patients [13.4%] in the glutamine treatment group and 20 patients [18%] in the placebo treatment group.
- ACS: 19 patients [10.1%] in the glutamine treatment group and 24 patients [21.6%] in the placebo treatment group. Eight patients (4.3%) and 13 patients (11.7%) in the glutamine and placebo groups, respectively, had TEAEs of acute chest syndrome that were considered *severe*.

Serious AEs:

'Sickle cell anaemia with crisis' was the most common SAEs: 124 patients [66.3%] in the glutamine treatment group and 80 patients [72.1%] in the placebo treatment group. One patient in the glutamine group and 1 patient in the placebo group had SAEs of sickle cell anaemia with crisis that were considered *related to treatment.*

Other SAEs potentially related to SCD are considered to be the PTs acute chest syndrome (7.0% vs 18.9% of patients in the glutamine or placebo groups, respectively), pyrexia (2.7% vs 3.6%), and pneumonia (including SOC infections and infestations) (4.8 vs 9.0%).

iii) Post-hoc analyses

Please refer to the section on analysis performed across trials later on.

Subgroup analyses

The Applicant provided subgroup analyses for regions, prior HU use, sex and age.

Efficacy across Regions

Subgroup analysis for efficacy across geographic regions was consistent across the subgroups studied, favoring L-glutamine for the number of sickle cell crises through Week 48 in all but one geographic regions (according to the point estimates of NBR analysis).

Applying NBR analysis with multiple imputation based on placebo completers on the dataset yielded similar findings.

Efficacy across Prior Hydroxyurea Use

'Prior' use was defined as patients who were receiving hydroxyurea at study entry. In both subgroups, the median number of sickle cell crises was lower through Week 48 in the L-glutamine-treated patients (medians

of 3 vs. 4 crises in both subgroups), but these differences were not statistically significant. A similar result was observed for number of hospitalizations (medians 2 vs. 3 in both subgroups), although for patients who had received prior hydroxyurea the difference was marginally significant (p=0.046). The results through Week 24 were similar for both endpoints.

Table 12: Number of SCC and hospitalizations for sickle cell pail by Hydroxyurea use through 24 and 48 weeks (Intent-to-Treat Population)

	Prior hydroxyurea use		No prior hydroxyurea use	
Variable and Interval	L-glutamine (N=101)	Placebo (N=52)	L-glutamine (N=51)	Placebo (N=26)
Number of Sickle Cell Crises				
Through Week 48				
Mean (SD)	3.3 (2.42)	4.0 (2.67)	3.0 (1.87)	3.7 (2.25)
Median	3	4	3	4
Range	0-15	0-15	0-11	0-11
	p<0.001[a] p=0.156[b]		p<0.001[a] p=0.147[b]	
Through Week 24				
Mean (SD)	1.8 (1.64)	2.3 (1.79)	1.6 (1.48)	1.8 (1.47)
Median	2	2	2	2
Range	0-7	0-7	0-8	0-6
	p=0.255[a] p=0.190[b]		p=0.026[a] p=0.672[b]	
Number of Hospitalizations for Sickle Cell Pain				
Through Week 48				
Mean (SD)	2.4 (2.23)	3.3 (2.54)	2.1 (1.41)	2.4 (1.68)
Median	2	3	2	3
Range	0-14	0-13	0-7	0-5
	p<0.001[a] p=0.046[b]		p=0.002[a] p=0.604[b]	
Through Week 24				
Mean (SD)	1.2 (1.34)	1.8 (1.61)	1.1 (1.11)	1.2 (1.07)
Median	1	2	1	1
Range	0-6	0-6	0-6	0-3
	p=0.021[a] p=0.062[b]		p=0.045[a] p=0.824[b]	

Source: Tables 14.5.2, 14.5.2.1, 14.6.2 and 14.6.2.1
SD = standard deviation; Range = min - max
[a] P-values are from the Fisher's Exact Test, testing for any difference in the distributions between treatment groups.

Baseline disease characteristics were generally balanced between the 4 arms: Glutamine, Glutamine + HU, Placebo, Placebo + HU.

While it is acknowledged that subgroup analyses for HU use have been performed, it is noted that these results only give information about glutamine efficacy in patients being treated with HU *prior* to study inclusion. The Company additionally substantiated that HU use was largely stable during the study (or changes thereto balanced between treatment arms) and that this consequently did not impact the efficacy outcome.

Consistency across groups was confirmed applying NBR analysis with multiple imputation based on placebo completers on the dataset.

Efficacy across Sex

For both sexes, the median number of sickle cell crises through Week 48 was lower in the L-glutamine group than in the placebo group (medians of 3 vs. 4 crises in both subgroups). Similarly, for both sexes the median number of hospitalizations through Week 48 was lower in the L-glutamine group than in the placebo group (medians 2 vs. 3 in both subgroups). For males, the same general trend was observed for these two

endpoints through Week 24 although the median number of hospitalizations for the females at Week 24 was 1 in both treatment groups.

Table 13: Number of SCC and Hospitalizations for Sickle Cell Pain by Sex Through 24 and 48 Weeks (Intent-To-Treat Population)

	Males		Females	
Variable and Interval	L-glutamine (N=73)	Placebo (N=33)	L-glutamine (N=79)	Placebo (N=45)
Number of Sickle Cell Crises				
Through Week 48				
Mean (SD)	3.1 (2.59)	4.0 (2.11)	3.2 (1.90)	3.9 (2.82)
Median	3	4	3	4
Range	0-15	0-9	0-9	0-15
	p<0.001[a] p=0.065[b]		p<0.001[a] p=0.732[b]	
Through Week 24				
Mean (SD)	1.7 (1.63)	2.5 (1.68)	1.8 (1.55)	1.8 (1.66)
Median	2	2	2	2
Range	0-8	0-6	0-6	0-7
	p=0.011[a] p=0.084[b]		p=0.014[a] p=0.927[b]	
Number of Hospitalizations for Sickle Cell Pain				
Through Week 48				
Mean (SD)	2.1 (2.11)	3.3 (2.21)	2.4 (1.87)	2.7 (2.38)
Median	2	3	2	3
Range	0-14	0-9	0-8	0-13
	p<0.001[a] p=0.038[b]		p=0.011[a] p=0.842[b]	
Through Week 24				
Mean (SD)	1.1 (1.19)	2.1 (1.64)	1.3 (1.33)	1.3 (1.25)
Median	1	2	1	1
Range	0-6	0-6	0-6	0-6
	p=0.003[a] p=0.016[b]		p=0.089[a] p=0.896[b]	

Source: Tables 14.5.3, 14.5.5, 14.6.3 and 14.6.5
SD = standard deviation; Range = min - max
[a] P-values are from the Fisher's Exact Test, testing for any difference in the distributions between treatment groups.
[b] P-values are from the Cochran-Mantel-Haenszel test, testing for a shift in the distributions between

Subgroup analyses separating sexes show that there is a trend towards a better efficacy response in males. With reference to the discussion below, it may be that these gender-related observations are confounded by differences in body weight and thus doses administered.

Consistency across sexes was confirmed applying NBR analysis with multiple imputation based on placebo completers on the dataset.

Efficacy across Age

For both age subgroups (children (patients ≤ 18 years) and adults (patients > 18 years)), the median number of sickle cell crises through *Week 48* was lower in patients in the L-glutamine treatment group than in patients in the placebo group (median 3 crises vs. 4 crises in both age groups). Similarly, the median number of hospitalizations through Week 48 was lower in patients in the L-glutamine treatment group compared to patients in the placebo group (2 vs. 3 in both age groups). There was less evidence of any difference between the treatment groups for these two endpoints through *Week 24* of treatment.

Table 14: Number of Sickle Cell Crises and Hospitalizations for Sickle Cell Pain by Patient Age Trough 24 and 48 Weeks (Intent-To-Treat Population)

Variable and Interval	Patients ≤ 18 years L-glutamine (N=75)	Patients ≤ 18 years Placebo (N=43)	Patients > 18 years L-glutamine (N=77)	Patients > 18 years Placebo (N=35)
Number of Sickle Cell Crises				
Through Week 48				
Mean (SD)	3.1 (2.07)	3.3 (2.00)	3.2 (2.42)	4.6 (2.94)
Median	3	4	3	4
Range	0-9	0-8	0-15	0-15
	p<0.001[a] p=0.802[b]		p=0.001[a] p=0.018[b]	
Through Week 24				
Mean (SD)	1.7 (1.56)	1.8 (1.31)	1.7 (1.62)	2.5 (2.03)
Median	2	2	2	2
Range	0-6	0-6	0-8	0-7
	p=0.058[a] p=0.681[b]		p=0.068[a] p=0.103[b]	
Number of Hospitalizations for Sickle Cell Pain				
Through Week 48				
Mean (SD)	2.3 (1.87)	2.6 (2.02)	2.3 (2.11)	3.4 (2.59)
Median	2	3	2	3
Range	0-8	0-6	0-14	0-13
	p<0.001[a] p=0.469[b]		p<0.001[a] p=0.025[b]	
Through Week 24				
Mean (SD)	1.2 (1.31)	1.3 (1.17)	1.1 (1.23)	2.0 (1.72)
Median	1	1	1	2
Range	0-6	0-4	0-6	0-6
	p=0.172[a] p=0.533[b]		p=0.006[a] p=0.049[b]	

Source: Tables 14.5.4, 14.5.6, 14.6.4, and 14.6.6
SD = standard deviation; Range = min - max
[a] P-values are from the Fisher's Exact Test, testing for any difference in the distributions between treatment groups.

The mean treatment difference (placebo vs. glutamine) was almost non-existent (i.e. 0.2 SCC) in the younger patients. The same trend was observed for the week 24 primary endpoint as well as for the number of hospitalisations at week 48 and 24. This strongly questions the beneficial effect of glutamine in this subgroup. SCD is a genetic disorder and children comprise a relevant target population. As for the gender-related differences discussed above, there was concern that the marginal treatment effect in patients <18 years could be due to an inadequate low dose. However, subgroup analyses using weight-based dose categories as factor did not further confirm this concern.

In NBR analysis with multiple imputation based on placebo completers on the dataset in the age group of 13-18 years of age, the results favoured placebo (rate ratio = 1.36 [0.91; 2.03]). Analyses based on age categories as well as on age as a continuous variable (using NBR analyses with multiple imputation based on the placebo group) revealed a significant treatment-by-age interaction. The observed "detrimental" effect in adolescents cannot be clearly dispelled by results of the phase II study, however, its biological plausibility seems difficult to explain.

Efficacy across baseline SCC rate cohorts

Additional analyses were requested to elucidate whether baseline SCC rate impacted efficacy. Whereas point estimates shifted in absolute terms as one would expect, it was found that rate ratios were largely consistent across the cohorts compared (see below).

Table 15: NBR Analysis of Number of SCCs at Week 48 by SCCs in Prior Year (2 SCCs, 3-5 SCCs, ≥6 SCCs) – Study GLUSCC09-01 (ITT Population)

Categories Statistics	Glutamine (N = 152)	Placebo (N = 78)
2 SCCs in prior year		
n	53	26
Rate Per 48 Weeks (95% CI)	2.57 (1.99, 3.33)	2.95 (2.10, 4.13)
Rate Ratio (95% CI)	0.87 (0.58, 1.33)	
3-5 SCCs in prior year		
n	76	36
Rate Per 48 Weeks (95% CI)	3.19 (2.56, 3.99)	4.28 (3.27, 5.61)
Rate Ratio (95% CI)	0.74 (0.53, 1.04)	
≥6 SCCs in prior year		
n	22	16
Rate Per 48 Weeks (95% CI)	4.77 (3.44, 6.63)	5.85 (4.01, 8.53)
Rate Ratio (95% CI)	0.82 (0.50, 1.34)	
p-value for H_0: No treatment by SCCs in Prior Year Group Interaction	p = 0.8397	

CI = confidence interval, H_0 = null hypothesis, ITT = intent-to-treat, N = total number of patients, NBR = negative binomial ratio, SCC = sickle cell crisis.

Summary of main study

The following table summarise the efficacy results from the main study supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 9: Summary of efficacy for trial GLUSCC09-01.

Title: A Phase III, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study of L-Glutamine Therapy for Sickle Cell Anemia and Sickle ß0-Thalassemia.		
Study identifier	GLUSCC09-01	
Design	Patients with sickle cell anemia (homozygous SS) and sickle $ß^0$-thalassemia were randomised in a 2:1 ratio to L-glutamine or placebo. The study included a screening period (up to 4 weeks prior to Week 0), a 48-week treatment period and a 3-week tapering period followed by a 2-week follow-up period for a maximum total of 57 weeks. Main efficacy objectives of study GLUSCC09-01 were to evaluate the efficacy of oral L-glutamine, as evaluated by the number of occurrences of sickle cell crises trough week 48 (primary analysis), the frequency of hospitalizations for sickle cell pain, the frequency of emergency room/medical facility visits for sickle cell pain, and by hematological parameters (hemoglobin, hematocrit, reticulocyte count) (secondary analyses).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	48 weeks not applicable not applicable
Hypothesis	Superiority over placebo	
Label	Prevention of sickle cell crises in adults and children older than 5 years suffering from sickle cell disease	

Treatments groups	L-Glutamine		L-Glutamine, 48 weeks, 151 randomized
	Placebo		Placebo, 48 weeks, 78 randomized
Endpoints and definitions	Primary endpoint	Number of sickle cell crises through Week 48 and prior to start of taper	
	Secondary endpoints	Number of sickle cell crises at Week 24; Number of hospitalizations for sickle cell pain at Weeks 24 and 48; Number of emergency room/medical facility visits for sickle cell pain at Weeks 24 and 48; Haematological parameters (haemoglobin, haematocrit, reticulocyte count).	
Database lock	04 MAR 2014		
Results and Analysis			
Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Primary Endpoint: Number of Sickle Cell Crises	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Rate (95% CI)	3.54 (3.16, 3.96)	3.97 (3.44, 4.59)
	Rate ratio (95.5% CI)	0.89 (0.75, 1.06)	
	Statistic test	Negative Binomial Regression Analysis, controlling for stratification variables 'region' and 'baseline hydroxyurea use' Missing data imputation: Single imputation, SCC count for all withdrawals was replaced by the mean number of SCCs for placebo completers, or actual SCC count (whichever was higher)	
	P-value	0.193	
Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Primary Endpoint: Number of Sickle Cell	Treatment group	L-Glutamine	Placebo

Crises	Number of subjects	152	78
	Rate ratio (95.5% CI)	0.82 (0.65, 1.04)	
	Statistic test	Negative Binomial Regression Analysis, controlling for stratification variables 'region' & 'baseline hydroxyurea use'. Missing data imputation: Multiple imputation analysis based on placebo completers	
	P-value	0.092	
Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Secondary Endpoint: Number of hospitalisations	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Rate ratio (95% CI)	0.82 (0.62, 1.08)	
	Statistic test	Negative Binomial Regression Analysis, controlling for stratification variables 'region' & 'baseline hydroxyurea use'. Missing data imputation: Multiple imputation analysis based on placebo completers	
	P-value	0.156	
Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Secondary Endpoint: Emergency room visits	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Rate ratio (95% CI)	0.75 (0.51, 1.10)	
	Statistic test	Negative Binomial Regression Analysis, controlling for stratification variables 'region' & 'baseline hydroxyurea use'. Missing data imputation: Multiple imputation analysis based on placebo completers	
	P-value	0.142	

Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Cumulative duration of crises (not pre-specified EP)	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	mean days (SD)	24.0 (25.84)	28.1 (33.16)
	Rate ratio (95% CI)	0.88 (0.63, 1.24)	
	Negative Binomial Regression Analysis, controlling for stratification variables 'region' & 'baseline hydroxyurea use'. Missing data imputation: Multiple imputation analysis based on placebo completers		
	p-value	p = 0.464	
Analysis description	**Newly requested analysis: NBR**		
Analysis population and time point description	Intent to treat population, Week 48		
Acute Chest Syndrome (part of the primary composite endpoint 'SCC')	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Rate ratio (95% CI)	0.41 (0.18, 0.92)	
	Statistic test	Negative Binomial Regression Analysis, controlling for stratification variables 'region' & 'baseline hydroxyurea use'. Missing data imputation: Multiple imputation analysis based on placebo completers	
	P-value	0.031	
Analysis description	**Pre-specified analysis, CMH test**		
Analysis population and time point description	Intent to treat population, Week 48		
Primary Endpoint: Number of Sickle Cell Crises	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78

	Mean (SD)	3.2 (2.25)	3.9 (2.53)
	Median	3	4
	Range	0-15	0-15
	Statistic test	Cochran-Mantel-Haenszel test, controlling for stratification variables 'region' and 'baseline hydroxyurea use' Missing data imputation: single imputation based on mean values of completers of the respective treatment arm	
	P-value	0.063	
Notes	The statistical test applied does not enable the quantification of the treatment effect by appropriate interval estimates.		
Analysis description	**Pre-specified analysis, CMH test**		
Analysis population and time point description	Intent to treat population, Week 48		
Secondary Endpoint: Number of Hospitalisations	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Mean (SD)	2.3 (1.99)	3.0 (2.31)
	Median	2	3
	Range	0-6	0-6
	Statistic test	Cochran-Mantel-Haenszel test, controlling for stratification variables 'region' and 'baseline hydroxyurea use' Missing data imputation: single imputation based on mean values of completers of the respective treatment arm	
	P-value	0.041	
Analysis description	**Pre-specified analysis, CMH test**		
Analysis population and time point description	Intent to treat population, Week 48		
Secondary Endpoint: Number of emergency	Treatment group	L-Glutamine	Placebo

room visits for sickle cell pain	Number of subjects	152	78
	Mean (SD)	1.1 (1.49)	1.6 (2.30)
	Median	1	1
	Range	0-12	0-15
	Statistic test	Cochran-Mantel-Haenszel test, controlling for stratification variables 'region' and 'baseline hydroxyurea use' Missing data imputation: single imputation based on mean values of completers of the respective treatment arm	
	P-value	0.128	
Analysis description	**Post-hoc analyses**		
Analysis population and time point description	Intent to treat population		
Hematological parameter: hematocrit, hemoglobin, reticulocytes	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	There were no significant differences between the groups in the hematological parameters at week 48 or week 24.		
Analysis description	**Post-hoc analyses**		
Analysis population and time point description	Intent to treat population		
Primary Efficacy Endpoint: Number of SCCs	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Mean (SD)	3.2 (2.24)	3.9 (2.54)
	P-value	0.0052	
	Statistic test	CMH test using modified ridit scores, controlling for region and HU use, *single imputation based on mean values of completers of the respective treatment arm*	
Analysis description	**Post-hoc analyses**		

Analysis population and time point description	Intent to treat population		
Primary Efficacy Endpoint: Number of SCCs	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Mean (SD)	3.25 (2.76, 3.82)	4.19 (3.44, 5.11)
	P-value	0.0366	
	Statistic test	NBR, no imputation	
Analysis description	**Post-hoc analyses**		
Analysis population and time point description	Intent to treat population		
Secondary Endpoint: number of hospitalisations	Treatment group	L-Glutamine	Placebo
	Number of subjects	152	78
	Mean (SD)	2.3 (1.99)	3.0 (2.33)
	P-value	0.0045	
	Statistic test	CMH test using modified ridit scores, controlling for region and HU use, single imputation based on mean values of completers of the respective treatment arm	

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

iii) Post-hoc analyses (using a CMH test with modified ridit scores).

As the pre-specified efficacy analyses according to study protocol did not allow concluding on study success, a new dataset was created and a 'new' analysis was performed. The Applicant argues that the rules and conventions applied here may differ from those used in the individual studies, but were employed for consistency, completeness, and/or because these rules were considered more clinically meaningful. Additional analyses were performed to aid in interpretation and understanding of the results, to provide sensitivity testing, and to provide robust evidence of efficacy by using alternative methods of analysis.

Analyses were performed a long time after completion of the pivotal phase III study, and a data-driven approach to present data cannot entirely be excluded. Results have to be interpreted with reference to the analysis that was specified a priori in the protocol and analysis plan.

Additional efficacy endpoints

Additional endpoints in *study GLUSCC09-01*:

Table 1. Efficacy Endpoints for Study GLUSCC09-01 in CSR and SCE

Endpoint	CSR for Pivotal Study GLUSCC09-01	SCE
Included in the SCE		
SCCs through Week 48 and prior to taper	Primary	Primary/Sensitivity
Rate of SCCs per 48 weeks	Not done	Sensitivity
Time to first SCC prior to taper	Additional	Other analysis of primary
Time to second SCC prior to taper	Not done	Other analysis of primary
SCC recurrent events prior to taper	Not done	Other analysis of primary
Number of SCCs by time of withdrawal for noncompleters	Not done	Other analysis of primary
Occurrences of acute chest syndrome (ACS) through Week 48 and prior to taper	Additional	Key secondary
Hospitalisations for sickle cell pain through Week 48	Secondary	Key secondary
Emergency room visits for sickle cell pain through Week 48	Secondary	Key secondary
Cumulative days in hospital during treatment phase	Additional	Other secondary/ additional
Haematologic parameters (haemoglobin, haematocrit, reticulocyte count)	Secondary	Other secondary/ additional
Blood transfusions through Week 48 and prior to taper	Not done	Other secondary/ additional
Not included in the SCE		
SCC through Week 24	Secondary	--
Hospitalisations for sickle cell pain though Week 24	Secondary	--
Emergency room visits for sickle cell pain through Week 24	Secondary	--
Height, weight, standard growth curve	Other	--

ACS = acute chest syndrome, CSR = Clinical Study Report, SCC = sickle cell crisis, SCE = Summary of Clinical Efficacy.

Additional endpoints in *study 10478*: 'rate of SCCs', 'time to first SCC' and 'blood transfusions'.

Statistical method for re-analysis of efficacy endpoints

Study GLUSCC09-01

Table 2. Analyses Performed for Study GLUSCC09-01 on the Primary Efficacy Parameter Data from the ISE Database

Analysis	Variable	Statistical Methodology	Imputation Method	Stratification Factors
Primary	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	Region[b] and hydroxycarbamide use
Sensitivity Analyses				
Omission of region as stratification factor	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	Hydroxycarbamide use
Omission of hydroxycarbamide use as stratification factor	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	Region[b]
Omission of both stratification factors	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	None
Ranking prior to analysis	Rank[c] of number of SCCs	CMH with tables as scores	CSR imputation rules[a]	Region[b] and hydroxycarbamide use
Alternative imputation methods	Number of SCCs	CMH with modified ridit scores	LOCF	Region[b] and hydroxycarbamide use
Alternative imputation methods	Number of SCCs	CMH with modified ridit scores	Time-adjusted LOCF	Region[b] and hydroxycarbamide use
Impact of ACS	Number of SCCs (excluding ACS)	CMH with modified ridit scores	CSR imputation rules[a]	Region[b] and hydroxycarbamide use
Alternative statistical methodology	Number of SCCs	NBR	None	Region[b] and hydroxycarbamide use
Other Analyses of SCCs				
Time to first and second SCC	Time to SCC[d]	Kaplan-Meier method, and Log-Rank test	None	None
Recurrent crisis event analysis	Time of each SCC	Andersen-Gill and Lin, Wei, Yang and Ying methods	None	None
Number of SCCs at time of withdrawal for noncompleters	Number of SCCs	Descriptive statistics	None	None

ACS = acute chest syndrome, CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ISE = Integrated Summary of Efficacy, LOCF = last observation carried forward, NBR = negative binomial regression, SCCs = sickle cell crises.

[a] Patients that discontinued prior to 48 weeks had their number of SCCs imputed as the larger of (1) the mean number of crises to the nearest integer for completed patients of the same treatment group, or (2) the actual number of crises at the time of discontinuation.

[b] Study sites were grouped into geographic regions prior to unblinding.

[c] Rank was determined without regard to treatment or strata.

[d] Patients with no SCCs were censored in the time to first SCC analysis and those with less than 2 SCCs were censored in the time to second SCC analysis. The censoring date was set to the earlier of their taper period start and last date on study medication.

The primary efficacy data were re-analysed using the originally intended CMH test with modified ridit scores and the same stratification factors as in the CSR. The Applicant states that this approach is consistent with the original intention of using the same inference procedure for estimating the study sample size and for the final analysis.

The following additional sensitivity analyses were performed to address the robustness of the primary efficacy analysis: Omitting randomization stratification factors, ranking prior to analysis, alternative imputation methods, alternative analysis of SCC (Removal of ACS), alternative statistical methodology (NBR), time- to-event analysis.

Table 3. Analyses Performed for Study GLUSCC09-01 on the Key Secondary Efficacy Parameter Data from the ISE Database

Analysis	Variable	Statistical Methodology	Imputation Method	Stratification Factors
Key Secondary				
ACS	Number of occurrences of ACS	CMH with modified ridit scores	None	Region[b] and hydroxycarbamide use
Hospitalisations	Number of hospitalisations	CMH with modified ridit scores	CSR imputation rules[a]	Region[b] and hydroxycarbamide use
ER visits	Number of ER visits	CMH with modified ridit scores	CSR imputation rules[a]	Region[b] and hydroxycarbamide use
Sensitivity Analyses				
Alternative imputation methods	Number of hospitalisations and ER visits	CMH with modified ridit scores	LOCF	Region[b] and hydroxycarbamide use
Alternative imputation methods	Number of hospitalisations and ER visits	CMH with modified ridit scores	Time-adjusted LOCF	Region[b] and hydroxycarbamide use
Alternative statistical methodology	Number of hospitalisations and ER visits	NBR	None	Region[b] and hydroxycarbamide use

ACS = acute chest syndrome, CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ER = emergency room, ISE = Integrated Summary of Efficacy, LOCF = last observation carried forward, NBR = negative binomial regression, SCCs = sickle cell crises.

[a] Patients that discontinued prior to 48 weeks had their number of hospitalisation or ER visits imputed as the larger of (1) the mean number to the nearest integer for completed patients of the same treatment group, or (2) the actual number at the time of discontinuation.

[b] Study sites were grouped into geographic regions prior to unblinding.

Study 10478

Table 5. Analyses Performed for Study 10478 on the Primary and Key Secondary Endpoints from the ISE Database

Analysis	Variable	Statistical Methodology	Imputation Method	Stratification Factors
Primary	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	Study site (centre)
Sensitivity Analyses				
Omission of stratification factor	Number of SCCs	CMH with modified ridit scores	CSR imputation rules[a]	None
Ranking prior to analysis	Rank[b] of number of SCCs	CMH with tables as scores	CSR imputation rules[a]	Study site (centre)
Alternative imputation methods	Number of SCCs	CMH with modified ridit scores	LOCF	Study site (centre)
Alternative imputation methods	Number of SCCs	CMH with modified ridit scores	Time-adjusted LOCF	Study site (centre)
Alternative statistical methodology	Number of SCCs	NBR	None	Study site (centre)
Other Analyses of SCCs				
Time to first SCC	Time to SCC[c]	Kaplan-Meier method, and Log-Rank test	None	None
Analyses of Key Secondary Parameters				
Hospitalisations	Number of hospitalisations	CMH with modified ridit scores	CSR imputation rules[a]	Study site (centre)
ER visits	Number of ER visits	CMH with modified ridit scores	CSR imputation rules[a]	Study site (centre)

CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ER=emergency room, ISE = Integrated Summary of Efficacy, LOCF = last observation carried forward, NBR = negative binomial regression, SCCs = sickle cell crises.

[a] For patients who discontinued with fewer than 85 days on treatment, the number of SCCs at Week 48 was imputed as the mean number of crises to the nearest integer for completed patients of the same treatment group. For patients who discontinued with 85 or more days on treatment, the number of crises at Week 48 was imputed as the actual number of crises at the time of discontinuation (whichever was larger). The same rules were used for number of hospitalisations and number of ER visits.

[b] Rank was determined without regard to treatment or stratum.

[c] Patients with no SCCs were censored. The censoring date was set to the earlier of their taper period start and last date on study medication.

Results

Results referring to the primary endpoints in both studies and on single results considered to be clinically meaningful are presented.

Study GLUSCC09-01

The results of the primary analysis demonstrated statistically significantly fewer (p = 0.0052) SCCs in favour of the glutamine group relative to placebo. The median number of SCCs was 25% less in the glutamine group than the placebo group or 1 SCC lower (Table 10).

Table 10. CMH Analyses of the Number of SCCs Per 48 Weeks - Study GLUSCC09-01 (ISE ITT Population)

	Glutamine N = 152	Placebo N = 78
Number of SCCs per 48 weeks		
Mean (SD)	3.2 (2.24)	3.9 (2.54)
Median (min, max)	3.0 (0, 15)	4.0 (0, 15)
P-value[a]	0.0052	
Frequency distribution of SCCs, n (%)		
0	15 (10)	4 (5)
1	16 (11)	10 (13)
2	17 (11)	11 (14)
3	62 (41)	4 (5)
4	16 (11)	23 (29)
5	8 (5)	12 (15)
6	6 (4)	5 (6)
7	5 (3)	4 (5)
8	2 (1)	2 (3)
9	3 (2)	1 (1)
11	1 (1)	1 (1)
15	1 (1)	1 (1)

CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ISE = Integrated Summary of Efficacy, ITT = intent-to-treat, max = maximum, min = minimum, SCCs = sickle cell crises, SD = standard deviation.
[a] Analyzed by CMH test with modified ridit scores controlling for CSR stratification factors (region and hydroxycarbamide use) and utilising CSR imputation rules.
Source: ISE Table 1.1.

Sensitivity analyses

The results of all sensitivity analysis were similar to the results for the primary endpoint and statistically significantly in favour of glutamine, for the 48-week time point.

NBR Analyses of the Rate of SCCs per 48 Weeks

Similarly to study GLUSCC09-01, a statistical model taking different observation periods of subjects into account and enabling quantification of the treatment effect by appropriate interval estimates (e.g. confidence intervals of the sickle count rate ratio that account for the group sequential testing and have a coverage of at least 95%) should be chosen.

The results of this analysis (including the CSR stratification factors in the model) demonstrated a lower rate of SCCs in the glutamine group relative to the placebo group (p = 0.0366) with a rate ratio (0.78) in favour of patients that received glutamine.

Table 15. NBR Analyses of the Rate of SCCs Per 48 Weeks - Study GLUSCC09-01 (ISE ITT Population)

NBR Modeling Results	Glutamine N = 152	Placebo N = 78
Rate of SCCs[a]		
Rate per 48 weeks (95% CI)	3.25 (2.76, 3.82)	4.19 (3.44, 5.11)
Rate ratio[b] (95% CI)	0.78 (0.61, 0.98)	
P-value[c]	0.0366	

CI = confidence interval, ISE = Integrated Summary of Efficacy, ITT = intent-to-treat, NBR = negative binomial regression, SCCs = sickle cell crises.

[a] The NBR model took time on study into account by transforming the number of events into rates.

[b] Rate ratio is (rate per 48 weeks for glutamine)/(rate per 48 weeks for placebo). A rate ratio < 1 favours glutamine.

[c] Analyzed by NBR (no imputation) including treatment, region, and hydroxycarbamide use as main effects and log (time on study) as an offset.

Source: ISE Table 1.1.6.

Key Secondary Analyses

Occurrences of ACS

ACS represents a serious complication and is one of the major reasons for hospitalisation and a major cause of mortality. The results of this analysis demonstrated statistically significantly fewer (p = 0.0028) occurrences of ACS in the glutamine group relative to placebo.

Table 18. CMH Analyses of Number of Occurrences of ACS per 48 Weeks - Study GLUSCC09-01 (ISE ITT Population)

	Glutamine N = 152	Placebo N = 78
Number of occurrences of ACS per 48 weeks		
Mean (SD)	0.1 (0.37)	0.3 (0.63)
Median (min, max)	0 (0, 2)	0 (0, 3)
P-value (controlling for region and hydroxycarbamide use)	0.0028	
Number of ACS occurrences, n (%)	13 (8.6)	18 (23.1)
0	139 (91)	60 (77)
1	10 (7)	13 (17)
2	3 (2)	4 (5)
3	0	1 (1)

ACS = acute chest syndrome, CMH = Cochran-Mantel-Haenszel, CSR = Clinical Study Report, ISE = Integrated Summary of Efficacy, ITT = intent-to-treat, SD = standard deviation, max = maximum, min = minimum.
Analyzed by CMH test with modified ridit scores and CSR stratification factors (controlling for region and hydroxycarbamide use).
Source: ISE Table 1.5.

Hospitalisations and ER Visits for Sickle Cell Pain

There was no difference in ER visits for sickle cell pain between treatment groups. The results of the analysis of the number of hospitalisations demonstrated statistically significantly fewer (p = 0.0045) hospitalisations for sickle cell pain in favour of the glutamine group relative to placebo. The median number of hospitalisations for sickle cell pain was approximately 33% less in the glutamine group than the placebo group (or 1 less hospitalization).

Other Pre-specified Secondary and Additional Efficacy Analyses

The median number of days in hospital was statistically significantly shorter (p = 0.022) in the glutamine group with 6.5 days compared to 11 days in the placebo group.

In the glutamine group, 47.4% of patients had at least 1 simple transfusion compared to 51.3% in the placebo group. The percentage of patients with more than 3 simple transfusions was lower in the glutamine group (12.5%) compared to placebo (24.4%). 2% of patients in the glutamine group had at least 1 exchange transfusion compared to 6.4% in the placebo group.

Study 10478

Although there was a tendency toward fewer SCCs in the glutamine group compared to the placebo group (mean number of SCCs: 4.3 and 9.6, respectively), the between-group differences were not statistically significant (p = 0.1501).

Table 24. CMH Analyses of the Number of SCCs Per 48 Weeks - Study 10478 (ISE ITT Population)

	Glutamine N = 37	Placebo N = 33
Number of SCCs per 48 weeks		
Mean (SD)	4.3 (5.22)	9.6 (17.88)
Median (min, max)	4.0 (0, 27)	4.0 (0, 90)
P-value	0.1501	

CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ISE = Integrated Summary of Efficacy, ITT = intent-to-treat, max = maximum, min = minimum, SCCs = sickle cell crises, SD = standard deviation.

[a] Analyzed by CMH test with modified ridit scores controlling for the CSR stratification factor (controlling for centre) and utilising CSR imputation rules.

Source: ISE Table 2.1.

The results of the *sensitivity analyses* were similar; none of the analyses revealed statistically significant differences between treatment arms.

NBR Sensitivity Analysis

The results of the NBR analysis demonstrated a statistically significant lower rate of SCCs in the glutamine group relative to the placebo group (p = 0.0240) with a rate ratio (0.47) in favour of patients that received glutamine.

Clinical studies in special populations

In the main study GLUSCC09-01, the age ranged from 5 to 58 years. In study 10478, age ranged from 9 to 58 years. Thus no data from patients older than 58 years is available.

Supportive studies

Supportive efficacy data from phase II study 10478 was presented. In addition, efficacy results have been collected in four legacy studies.

Study 10478: A Phase II, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study of L-Glutamine Therapy for Sickle Cell Anemia and Sickle β0-Thalassemia

Phase 2, prospective, randomized, double-blind, placebo-controlled, parallel-group, multicentre study to evaluate the safety and efficacy of oral L-glutamine therapy for patients with sickle cell anaemia or sickle ß0-thalassemia who are at least 5 years old. Patients were randomized to L-glutamine or placebo in a 1:1 ratio

after a 4-week screening period. Patients underwent 48 weeks of treatment with dosing BID orally. Patient visits occurred every 4 weeks. After 48 weeks of treatment, dose was tapered to zero within 3 weeks. A final evaluation visit occurred 2 weeks after last dose. Clinical and haematological parameters were monitored for efficacy assessment, while the adverse events, especially those attributable to glutamine therapy, were also monitored.

Outcomes/endpoints

Primary and secondary objectives were generally similar to the ones of GLUSCC-0-01.

- Primary efficacy endpoint: number of painful sickle cell crises through Week 48 and prior to start of taper. A painful sickle cell crisis was defined as a visit to a medical facility that lasted more than 4 hours for acute sickling-related pain that was treated with a parenterally administered narcotic (except for facilities in which only orally administered narcotics were used).
- Secondary efficacy endpoints: number of hospitalisations for sickle cell pain, number of emergency room visits for sickle cell pain, days usual activities were interrupted due to sickle cell pain, height, weight, growth curve (< 18 years of age), hematologic parameters, narcotic usage, alcohol and tobacco use, pain level, energy level, activity level, appetite, subjective exercise tolerance, subject quality of life (RAND 36-Item Health Survey and Peds QL Pediatric Quality of Life Questionnaires).
- Safety endpoints: incidence of adverse events (AEs), safety laboratory results, and vital signs.

Sample size

The sample size was based on results from preliminary data and the literature, which showed that the mean number of painful sickle cell crises in a year was 6.5, with a standard deviation of 5.5. A sample size of 40 patients per treatment group would have 95% power to detect a difference in means of 4.5 (the difference between means of 2.0 for the Glutamine group and 6.5 for the placebo group), assuming a common standard deviation of 5.5 using a t-test with a 0.05 two-sided significance level.

Randomisation

Randomisation of all patients at all sites was performed centrally by The Investigational Drug Service (IDS) of LA BioMed. They generated and implemented the randomization schedule and kept the record of study medication. Randomisation was by site using a 1:1 ratio of glutamine versus placebo. Randomisation was planned for approximately 80 patients (40 patients per treatment group).

Blinding

The investigators, site staff, patients, and study personnel were blinded to treatment assignment. Both Glutamine and placebo are white powders with an identical appearance. The placebo was equivalent in volume to the study medication. In addition, to ensure the blinding of the taste, the powders were mixed immediately before ingestion with a beverage or food for administration. For the interim analysis, the treatment blind was maintained for the final analysis by (a) having an independent statistician perform the analysis, and (b) using a series of protocol specific procedures (PSPs) to ensure maintenance of the blind.

Statistical methods

Statistical tests were performed against a two-sided alternative hypothesis, with a significance level of 5% (α =0.05). According to the protocol, center will be taken into account in all analyses. If reasonable, the data from low enrolling centers were planned to be combined. The decision how to combine centers was made

after all patients are enrolled and prior to unblinding the data. The methods for combining centers were documented.

The primary efficacy parameter was initially planned to be analysed using ANOVA, but was subsequently changed to a nonparametric approach, the Cochran-Mantel-Haenszel test, which was planned for a variety of other secondary parameters. The number of painful sickle cell crises through week 24, the number of hospitalizations, the number of emergency room visits for sickle cell pain, the percentage of days that a patient's daily activities are interrupted due to sickle cell pain, height and weight, alcohol usage, tobacco usage, pain level were also analysed using ANOVA. Ordinal variables were compared using a CMH test controlling for study centre.

Handling of Missing Values: For discontinued patients with less than 85 days on treatment, the number of crises was imputed by the mean number of crises for the completed patients of the same treatment group. For discontinued patients with 85 days or longer on treatment, the number of crises at Week 48 was imputed by patient according to the individual rate of crises at the date of withdrawal. All imputed values were rounded up to the nearest whole integer. The same method was also used for the secondary endpoints of number of crises at Week 24, number of hospitalizations for sickle cell pain at Weeks 24 and 48, and number of emergency room visits for sickle cell pain at Weeks 24 and 48. This determination was documented prior to release of the randomization. Overall, the same issues on missing data imputation as discussed for study GLUSCC09-01 above apply.

The use of the non-parametric CMH approach instead of the initially planned ANOVA, and the slight adaptation of missing value handling (after having observed that more than half of the randomised patients did not complete the study, using a more or less arbitrary cut-off of 85 days to replace missing observations by the mean of the same treatment arm (≤ 85 days), or by "annualisation" by patient (>85 days)) are acknowledged. Since the revised analysis is very similar to the primary analysis of the confirmatory study GLUSCC09-01, the issues raised there apply here as well, in particular with respect to the estimand, the adjustment for observational period/treatment duration, the quantification of the treatment difference and the handling of missing values.

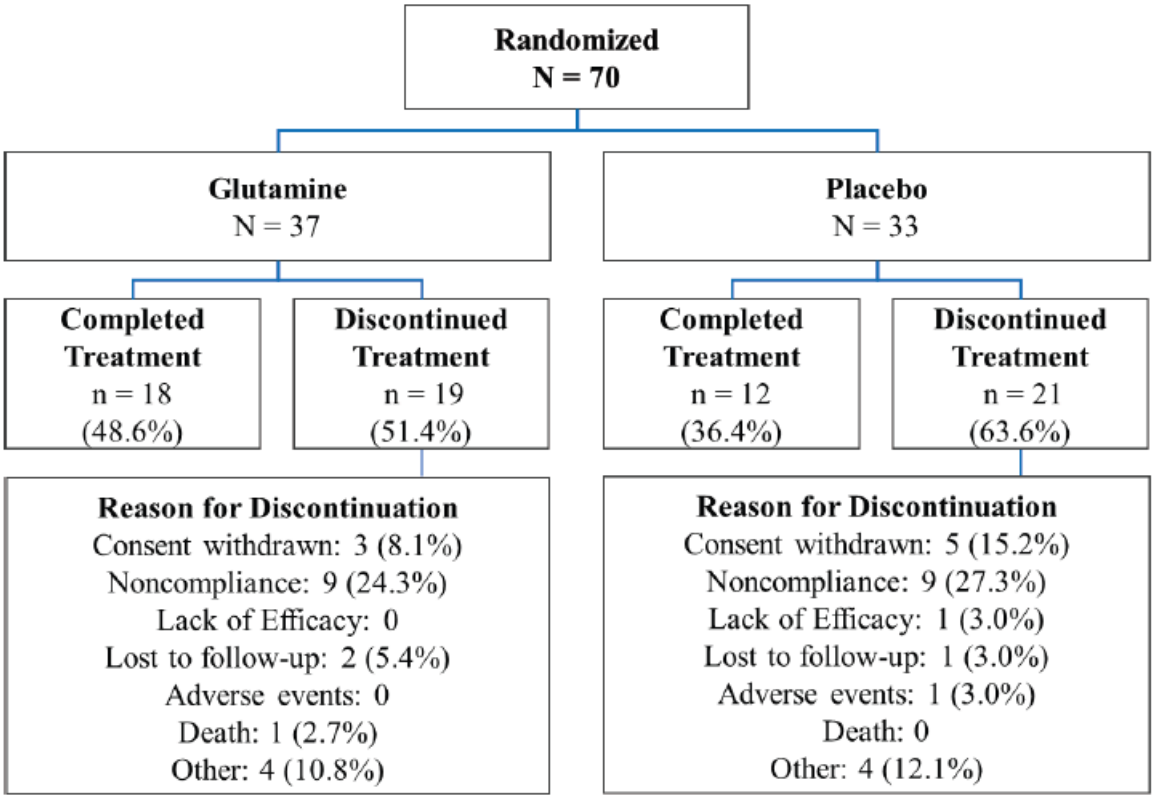
Overall, the results of the supportive study 10478 are considered for assessment in order to gain additional, independent evidence. Thus, the same methodological concerns were raised and additional discussion/analyses requested as for study GLUSCC09-01.

Results

Participants flow

A total of 70 patients were randomised in the study (intent-to-treat population). Of these, 37 patients were in the Glutamine group and 33 were in the placebo group. Patient disposition is summarised in the figure below.

Figure 5: Patient Disposition-Study 10478.



Outcomes and estimation

<u>i) Newly requested analyses using CMH & NBR analyses</u>

Primary (number of sickle cell crises) and secondary efficacy analyses

Under Imputation Scheme # 3, the CMH results were not significant (p = 0.1903) but the NBR results indicated a significantly lower rate of SCCs for glutamine than for placebo (rate ratio = 0.56, p = 0.012).

Table 46. SCCs with Imputation Scheme #3 – Study 10478

Descriptive Statistics	Placebo (N = 33)	Glutamine (N = 37)
Mean (SD)	8.4 (14.18)	4.7 (4.48)
Median	5.0	5.0
Min, Max	0, 82	0, 27
Number of crises (n)		
0	1	5
1	2	2
2	4	2
3	0	1
4	0	4
5	18	18
6	0	1
7	0	1
8	1	0
9	2	1
11	0	1
13	2	0
17	1	0
27	1	1
82	1	0

max = maximum, min = minimum, N = total number of patients, n = number of patients, SCC = sickle cell crisis, SD = standard deviation.

Source: Table Q100a.12

Table 47. CMH and NBR Analyses of Number of SCCs at Week 48 Using Imputation Scheme #3 – Study 10478

	Placebo (N = 33)	Glutamine (N = 37)
CMH test, controlling for centre and HU use		
p-value	0.1903	
NBR[a] Modeling Results		
Rate Per 48 Weeks (95% CI)	7.39 (5.17, 10.58)	4.15 (2.88, 5.99)
Rate Ratio[b] (95% CI)	0.56 (0.36, 0.87)	
p-value[c]	0.012	

Imputation Scheme #3 is based on the placebo group. The SCC count for all withdrawals will be the mean number of SCCs for placebo completers, or actual SCC count (whichever is higher).

CI = confidence interval, CMH = Cochran–Mantel–Haenszel test, H_0 = null hypothesis, HU = hydroxycarbamide, NBR = negative binomial regression, SCC = sickle cell crisis.

[a] NBR model with Treatment, Centre, and HU Use as main effects. One subject who was randomised but never took study medication was not included in the analysis.

[b] Rate ratio is (rate per 48 weeks for Glutamine)/(rate per 48 weeks for Placebo). A rate ratio < 1 favours Glutamine.

[c] p-value for H_0: No Difference Between Treatments.

Source: Table Q100a.12 and Table Q100a.16

Analyses using multiple imputation techniques (imputing missing observations based on data from placebo completers) and negative binomial regression showed a rate ratio of 0.45 after 48 weeks of treatment (95%CI: 0.22, 0.90, p=0.025) for the primary endpoint.

The results of a negative binomial regression analysis and multiple imputation based on placebo completers of rate of hospitalisations per 48 weeks showed a rate ratio (95%CI) of 0.67 (0.33, 1.35).

ii) Pre-specified analyses using a CMH test

The mean number of painful sickle cell crises through *Week 48* was 4.5 in the L-glutamine group compared to 10.8 in the placebo group but the difference was not statistically significant for the full analysis dataset (Table 11-3). The mean number of crises through *Week 24* was 2.5 in the L-glutamine group and 5.5 in the placebo group. The results were similar for the per-protocol dataset.

Table 11-3 Number of Painful Sickle Cell Crises

	Full Analysis Dataset		Per-protocol Dataset	
Interval	L-glutamine (N = 33)	Placebo (N = 29)	L-glutamine (N = 24)	Placebo (N = 19)
Through Week 48				
Mean (SD)	4.5 (5.37)	10.8 (18.74)	4.3 (5.99)	10.8 (20.61)
Median	4	5	4	4
Range	0 – 27	0 – 90	0 – 27	0 – 90
	p = 0.076 [a]		p = 0.117 [a]	
Through Week 24				
Mean (SD)	2.5 (2.55)	5.5 (8.46)	2.3 (2.87)	5.4 (9.05)
Median	2	3	2	2
Range	0 – 12	0 – 38	0 – 12	0 – 38
	p = 0.060 [a]		p = 0.102 [a]	

Source: Tables 14.2.2.1a and 14.2.2.1b

a P-values are from Cochran-Mantel-Haenszel tests controlling for study center.

Secondary Efficacy Endpoints: Number of Hospitalizations and Emergency Room Visits for Sickle Cell Pain

There were significantly fewer hospitalizations for sickle cell pain through *Week 24* in the L-glutamine group than in the placebo group, with means of 0.8 versus 1.3 for the full analysis dataset (p = 0.036; Table 11-4). A similar pattern was seen through *Week 48* (means of 1.5 versus 2.3 hospitalizations) (not statistically significant). For the per-protocol dataset the difference between treatment groups in favor of the L-glutamine group was statistically significant through Week 48 (p = 0.032), but not at week 24 (p=0.052).

There were fewer emergency room visits on average in the L-glutamine group compared to the placebo group through Week 24 and through Week 48 for both the full analysis dataset and the per-protocol dataset (not statistically significant) (Table 11-4).

Table 11-4 Number of Hospitalizations and Emergency Room Visits for Sickle Cell Pain

Variable and Interval	Full Analysis Dataset L-glutamine (N = 33)	Full Analysis Dataset Placebo (N = 29)	Per-protocol Dataset L-glutamine (N = 24)	Per-protocol Dataset Placebo (N = 19)
Number of hospitalizations for sickle cell pain				
Through Week 48				
Mean (SD)	1.5 (2.46)	2.3 (2.42)	0.9 (1.23)	2.4 (2.81)
Median	1	2	0	2
Range	0 – 10	0 – 10	0 – 4	0 – 10
	p = 0.072 [a]		p = 0.032 [a]	
Through Week 24				
Mean (SD)	0.8 (1.18)	1.3 (1.42)	0.5 (0.78)	1.3 (1.63)
Median	1	2	0	1
Range	0 – 5	0 – 5	0 – 3	0 – 5
	p = 0.036 [a]		p = 0.052 [a]	
Number of emergency room visits for sickle cell pain				
Through Week 48				
Mean (SD)	3.7 (5.63)	9.4 (19.91)	3.9 (6.53)	9.3 (22.03)
Median	2	3	2	2
Range	0 – 27	0 – 94	0 – 27	0 – 94
	p = 0.129 [a]		p = 0.188 [a]	
Through Week 24				
Mean (SD)	1.9 (2.71)	4.7 (9.02)	2.0 (3.13)	4.5 (9.73)
Median	1	2	1	1
Range	0 – 12	0 – 40	0 – 12	0 – 40
	p = 0.105 [a]		p = 0.186 [a]	

Source: Tables 14.2.2.2a, 14.2.2.2b, 14.2.2.3a, and 14.2.2.3b

a P-values are from Cochran-Mantel-Haenszel tests controlling for study center.

Secondary Efficacy Endpoint: Interruptions of Usual Daily Activities Due to Sickle Cell Pain

No between-group comparisons of the change from baseline over time were statistically significant and there was no consistent pattern between the treatment groups.

Secondary Efficacy Endpoint: Height, Weight, and Growth Curve

There were no clinically and statistically significant between-group differences.

Secondary Efficacy Endpoint: Narcotics Use

There were no statistically significant differences between the groups in narcotic usage. Within each group the results were the same at Week 24 and Week 48. However, relative to baseline, 35% of patients in the L-glutamine group (N = 17) changed to weaker narcotics, 59% had no change, and 6% changed to stronger narcotics. In the placebo group (N = 16), 13% of patients changed to weaker narcotics, 56% had no change, and 31% changed to stronger narcotics.

Secondary Efficacy Endpoint: Pain Level

There were no statistically significant between-group differences with regard to pain level and mean percentage of days with the worst pain.

iii) Post-hoc analyses

Please refer to the section on analysis performed across trials above.

Subgroup analyses

The 11 patients randomized at Site 106 were excluded from the primary analyses due to potential scientific misconduct at that site. The Applicant provided a narrative of the events leading to the discovery of investigator misconduct and fraud in Site 106, which is acceptable.

With these patients included, a total of 42 patients were randomized to L-glutamine and 39 to placebo (the safety population). The full analysis dataset included 38 and 35 patients in the L-glutamine and placebo groups, respectively. The demographic characteristics of the study population did not change markedly with the addition of these patients. Primary efficacy results including site 106 were in the same range of those excluding the site, a slight trend towards worse outcome visible (data from NBR, multiple imputation based on placebo completers). Overall, it appears that the exclusion of this site did not introduce any bias.

Legacy studies:

Additional efficacy results have been presented in 4 legacy studies.

Study 8288 (Niihara, *et al* 1998; Pilot Study)

This was an open-label, uncontrolled, single-center, pilot study conducted to evaluate the effect of L-glutamine treatment on NAD, NADH and NAD redox potential of sickle RBCs. Seven subjects (19 to 60 years old) with sickle cell anemia were treated with oral L-glutamine 30 g/day (10 g, TID) for 4 weeks. The total NADH level increased from 47.5 ± 6.3 nmol/mL RBC to 72.1 ± 15.1 nmol/mL RBC ($P < 0.01$), with the NAD redox potential increasing from 47.2 ± 3.7% to 62.1 ± 11.8% ($P < 0.01$) in study patients.

Study 8775

This was a Phase 2a, single-center, prospective, randomized, double-blind, placebo-controlled, crossover study designed to evaluate the effect of L-glutamine treatment on total NAD, NAD redox potential, RBC endothelial cell adhesiveness, hematologic parameters, frequency of painful crises, number of hospitalization day, number of painless days, and safety. In the 6 evaluable subjects, there was a significant increase in the number of painless days ($p = 0.00885$). There was a trend, but no statistically significant improvement in the number of painful crises observed ($p = 0.28$).

Study 10779

This was an open-label, controlled, single-centre study designed to evaluate the effect of L-glutamine treatment on subjects' subjective perception of clinical status, safety, and exercise endurance. Eight out of 14 subjects with sickle cell anaemia enrolled completed the study. Seven subjects participated in the exercise endurance studies. Five control subjects were enrolled. For the incremental work protocol, the work rates were 88 watts at baseline and 91.5 watts after treatment ($p = 0.18$), showing trend for increase with L-glutamine treatment. For the constant work protocol, the duration of exercise was 5.87 minutes at baseline and 7.27 minutes after treatment ($p = 0.02$), showing a significant improvement in the work rate after treatment with L-glutamine.

Study 10511

This was a Phase 2, single-centre, prospective, randomized, double-blind, placebo-controlled, parallel-group study conducted to evaluate the effect of L-glutamine treatment on exercise endurance, breath-by-breath exercise response, the incidence of painful crises, level of chronic pain, amount of daily requirement of narcotics, and safety. This study was terminated prior to achieving the planned enrolment due to inadequate

supplies of investigational product and internal business decisions. Due to this, the efficacy parameters were not analysed.

2.5.3. Discussion on clinical efficacy

Efficacy data supporting the application were collected in 6 clinical studies: the pivotal phase 3 Study GLUSCC09-01, supportive phase 2 study 10478 and 4 legacy clinical studies, the latest contributing with very limited additional information.

Design and conduct of clinical studies

Study GLUSCC09-01

The study consisted of a 4-week screening period, a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period.

230 patients were randomised in a 2:1 ratio to glutamine or placebo. Randomisation was stratified by investigational site (region) and prior hydroxyurea (HU) use (yes/no). Patients as well as investigators were blinded. During the 48-week treatment period, patients self-administered an equivalent volume of glutamine or placebo as oral powder twice daily. The daily dose was 10, 20 or 30 g, based on categorisation into three body weight categories, of <30 kg, 30-65 kg and >65 kg, respectively, with an upper limit of 30 g/day. After 48 weeks of treatment, patients were gradually tapered off study medication over a period of 3 weeks. The final visit was at Week 53, i.e., 2 weeks after the last dose.

The primary efficacy endpoint was the number of sickle cell crises through Week 48 and prior to start of taper. The definition of the primary endpoint was revised on 3 occasions after study start, i.e. after the first patient was enrolled, but the impact of this on the integrity of the study results of the placebo-controlled, double-blind study is likely minimal. The Applicant clarified that the definition of sickle cell crisis for the purpose of inclusion in the study was the same as the one used for crises count for primary efficacy analysis. This is endorsed and is considered relevant for an analysis including baseline crises count as a covariate.

An adjudication committee (CAC) was used to re-adjudicate SCCs determined by investigators upon patient admission. This was done unidirectionally (i.e. only a crisis judged by investigator as such could be re-adjudicated by CAC to 'no crisis'). The Applicant states that this re-adjudication was performed in a blinded fashion. Upon review, a noteworthy difference in re-adjudication rates (Xyndari vs. placebo) was noted, however. About twice as many SCCs occurring in the Xyndari arm were overruled (to 'no crisis') compared to placebo SCCs, and this imbalance remains unexplained.

Secondary efficacy endpoints included the number of sickle cell crises at Week 24, number of hospitalisations for sickle cell pain at Weeks 24 and 48; number of emergency room/medical facility visits for sickle cell pain at Weeks 24 and 48, and haematological parameters (haemoglobin, haematocrit, reticulocyte count).

The defined endpoints are considered relevant, but only monitored severe crises (crises needing parenteral narcotics, hospitalisations, etc.). Literature indicates that most of the crises in SCD patients are self-managed at home (e.g. by using oral opioids). No information on crisis occurrence and pain management at home is available and consequently, it cannot be verified whether crisis incidence changed overall or whether there was a mere shift in crisis severity from severe to self-manageable.

Phase 2 study 10478

This study was similarly designed as study GLUSCC09-01, i.e. multicentre, double-blind, randomised and placebo-controlled. 70 patients were randomised to either glutamine or placebo. Also the selected population was largely similar to the phase III trial.

The main differences compared to study GLUSCC09-01 are the 1:1 randomisation ratio and stratification only by investigational site (not by prior HU use). This resulted in a remarkable imbalance between treatment arms with regard to prior HU use, with 43.2% vs. 27.2% of patients being pre-treated with HU in the glutamine and placebo arms, respectively. This imbalance has to be kept in mind when interpreting study results.

The primary efficacy endpoint was the number of painful sickle cell crises through Week 48 and prior to start of taper. The definition slightly differs from the one used in study GLUSCC09-01: A painful sickle cell crisis was defined as a visit to a medical facility that lasted more than 4 hours, for acute sickling-related pain which was treated with a parenterally administered narcotic (except for facilities in which only orally administered narcotics are used). The measurement of the length of the visit included all time spent after registration at the medical facility, including time spent waiting to be seen by a physician. The occurrence of chest syndrome (chest-wall pain in association with findings of a new pulmonary infiltrate on chest x-ray films and fever), priapism, and hepatic or splenic sequestration was also considered a crisis.

Secondary efficacy endpoints included: Number of hospitalisations for sickle cell pain; number of emergency room visits for sickle cell pain; number of days patient's usual daily activities are interrupted due to sickle cell pain; height; weight; growth curves; haematological parameters (haemoglobin, haematocrit, reticulocyte count); narcotic usage; pain level, etc.

Thus, in contrast to study GLUSCC09-01, where only efficacy in terms of severe crises needing visits to medical facilities or even hospitalisation have been reported, some endpoints of this study also reported on pain management of milder crises. Especially narcotics use is seen as a relevant endpoint.

In study GLUSCC09-01, more patients discontinued in the glutamine as compared to the placebo arm: 64.2% (97/151) in the glutamine group compared to 75.6% (59/78) in the placebo group completed the study.

In study 10478, only 18 patients (48.6%) in the Glutamine arm and only 12 patients (36.4%) in the placebo arm completed the study.

The high and imbalanced number of withdrawals and thus the approach of dealing with missing data in the statistical method to assess the treatment effect has a major impact on the interpretation of study results.

Legacy studies

Only study 8775 and study 10779 provide efficacy results from a placebo-controlled setting. The number of evaluable patients was low in both trials (6 and 8 subjects, respectively). Furthermore, the statistical approach chosen for both studies has not been described in the dossier or seems inadequate (no comparison against placebo). Therefore, the results of these studies are not considered supportive.

Efficacy data and additional analyses

Study GLUSCC09-01

The pre-specified analysis has major limitations due to its statistical method including missing data imputation. Results after 48 weeks of treatment revealed a median of 3 vs. 4 sickle cell crises (primary

endpoint) in the glutamine and placebo groups, respectively. This difference was not statistically significant. The respective mean values were 3.2 vs. 3.9.

Nearly all patients with SCC had one or more events defined by the first component, i.e., a visit to an emergency room/medical facility for SCD-related pain, treated with a parental narcotic or toradol (ketorolac) or an oral narcotic.

A median of 2 vs. 3 hospitalisations was observed in the glutamine and the placebo groups, respectively (the respective mean values were 2.3 vs. 3.0).

There were no pronounced differences in haematological parameters (haematocrit, haemoglobin, reticulocytes) at week 24 or week 48.

The statistical method including the missing data imputation are considered major issues in the demonstration of efficacy. The analyses quantified the 48-week treatment difference assuming that patients adhered to the assigned treatment. This does not appropriately address treatment discontinuation, which occurred frequently and more so in the glutamine arm. It can generally not be assumed that the reason for study/treatment discontinuation occurs independent of drug intolerance or a perceived lack of efficacy. The estimand better reflecting clinical practice and thus of major interest to decide on patient benefit, is considered to be the effect within the 48 weeks period regardless of whether the patient adhered to treatment (treatment policy strategy). The Applicant was requested to make plausible and suitable (i.e. not favoring experimental treatment) assumptions for the study period after withdrawal as it turned out that post-treatment-withdrawal data were not recorded.

Furthermore, the application of the Cochran–Mantel–Haenszel test was questioned, because its interpretation depends upon the duration of drug exposure/follow-up and there were considerable differences in discontinuation rates between the treatment arms (36% vs 24% of patients in the glutamine and placebo arms, respectively). The corresponding estimates of the treatment difference are difficult to interpret, and a statistical model was requested that enables the quantification of the treatment effect and provides point- and interval estimates taking the sequential testing into account.

Lastly, and with reference to the above mentioned 'treatment policy strategy' the handling of missing data was criticised. The SCC count for patients discontinuing during the trial had been imputed using the mean number of crises for the patients of the same treatment group who did complete Week 48. This is not considered reasonable to quantify the 48-week treatment effect for subjects who withdraw early from glutamine treatment and may also lead to a substantial underestimation of the variability within treatment arms. Instead, for patients dropping-out during the study, the SCC number needs to be discounted for the non-treated period (e.g. based on the placebo group or based on patients whose efficacy data were recorded after treatment discontinuation), and the statistical method should take the uncertainty of the missing data into account (e.g. multiple imputation techniques).

The Applicant provided additional analyses, using negative binomial regression analysis with 4 different imputation schemes:

- scheme #1 imputing the maximum number of SCCs observed among completers in the same treatment arm;
- scheme #2 imputing the maximum number of SCCs observed among all completers;
- scheme #3 imputing the mean SCCs of placebo completers or actual SCC count (whichever is higher);

- and scheme #4 imputing the mean SCCs of all completers.

The estimated change in SCC varied greatly depending on the analysis and imputation methods used, being consistently small (to non-existent) in all the imputation schemes presented. Imputation scheme #3 is considered the most appropriate and using suitable assumptions in the analysis to inform on the benefits of glutamine. Under imputation scheme #3, the rate ratio favours glutamine (point estimate: 0.89, 95.5%CI: 0.75, 1.06), but the difference was not statistically significant (p = 0.193).

Of note, all of the 4 imputation schemes results were far from reaching statistical significance. The "worst case" scenarios (schemes 1 and 2) lead to inconclusive results, which do not support the hypothesis of a glutamine benefit in terms of SCCs: Increases of the SCC rate of up to 46% in the glutamine as compared to the placebo arm cannot be excluded considering the upper limit of the CIs.

Multiple imputation techniques were requested to reflect an appropriate degree of uncertainty. The rate ratio employing multiple imputation and based on data from placebo completers was 0.84 (95.5%CI: 0.66, 1.06; p=0.129).

Secondary endpoints (i.e. hospitalisation and ER visit rates) showed concordant results.

Additional analyses on ACS (without pre-specification but ACS being part of the primary composite endpoint) showed a rate ratio of 0.41 (95%CI: 0.18, 0.92). In the 'post-hoc analysis', performed years after finalisation of the study report, mean and median values were 0.1 vs. 0.3 and 0 vs. 0 in both groups, respectively. Other "major" SCD related events also part of the SCC definition (i.e. splenic sequestration, priapism) were not reduced.

Time-to-next sickle cell crisis (time to first and second SCC and recurrent crisis event time, evaluated post-hoc) generally indicated a longer protective effect under glutamine treatment compared to placebo. However, these endpoints are likely strongly correlated with the primary endpoint and are therefore expected to provide only marginal additional insight into the treatment effect of glutamine. These time-to-event analyses suffer from the same limitations as the count data analysis in terms of handling of missing data. Of greater interest is the cumulative time spent in crisis. Respective analyses where requested and results show a small effect in favour of glutamine (mean days: 24 vs. 28.1 for glutamine and placebo respectively, p=0.492). However, the difference was not statistically significant.

The Applicant provided subgroup analyses for regions, *prior* HU use, sex, age and baseline SCC count. Concern was raised that a dose below 30 g per day could be too low, as the treatment effect seems to be generally higher in males as compared to women as well as in adults as compared to children. However, the Applicant provided additional information on as well as analyses supporting the chosen dosing schedule and more generally dispelled the concern that the lower doses may not be adequate. There were inconsistent apparent effects in children and a difficult to interpret signal towards detrimental effect in adolescents. Some inconsistency in effect between regions was also observed.

With reference to the imbalanced re-adjudication of the CAC, additional analyses were requested using the dataset as adjudicated by investigators originally. For the primary endpoint as well as most secondary analyses, the point estimates shift even further towards 'no effect' in the investigator-adjudication dataset. For the primary endpoint, a rate ratio of 0.92 (95.5% CI: 0.74, 1.14; p = 0.448) is reported based on investigator-adjudicated data.

<u>Study 10478</u>

Results of the phase 2 study are broadly consistent with what was found for the pivotal phase 3 study. However, small study size and the impact of outliers as well as the imbalance of HU use at baseline (around 16% difference between treatment arms) complicate the interpretation of efficacy outcomes. Furthermore, the same issues with regard to the statistical analyses as for study GLUSCC09-01 apply and the dropout rate was even higher for this study (less than 50% of subjects completing the study).

In conclusion, in the pre-specified primary efficacy analysis, glutamine treatment was associated with a trend towards fewer sickle cell crises through Week 48 compared to placebo treatment. However, the results were not statistically significant, and the primary efficacy analysis thus failed.

'Post-hoc' analyses, partly yielding better results, were performed years after finalisation of the study reports and need to be interpreted with caution as a data-driven approach cannot entirely be excluded.

As indicated above, a large and imbalanced proportion of patients (more in the glutamine than in the placebo arm) discontinued the study and were apparently not followed, i.e., crises occurring after withdrawal have not been collected/counted. Consequently, the impact of missing data imputation is large, which increases the uncertainty of the estimation of the treatment effect. Additional efficacy analyses using NBR regression and different imputation schemes, requested based on concerns regarding the initially employed methodology, do not show a statistically significant treatment effect over placebo.

Furthermore, the estimated difference in SCC varies greatly depending on the analysis and imputation methods used, being consistently small in all imputation schemes presented by the Applicant. In the most realistic imputation scenario (scheme #3), the difference in the mean number of SCC was 0.4 and the risk ratio was 0.89.

Additionally, the imbalance between investigators and the CAC assessment with regard to the number of adjudicated crises, favouring the glutamine arm, is large and unexpected and potentially important bias cannot be excluded. Analyses based on the investigator-adjudicated SCC count are also considered relevant for understanding glutamine efficacy in SCD.

Overall, together with the fact that results from study 10478 provide only limited support, it is concluded that efficacy has not been convincingly demonstrated.

2.5.4. Conclusions on the clinical efficacy

Efficacy of Xyndari in reducing the frequency of SCCs has not been established. The single pivotal phase 3 study submitted failed the primary analysis pre-specified in the statistical analysis plan.

The following major issues were identified in study GLUSCC09-01 and remain unresolved:

- Notwithstanding the Applicant´s arguments regarding the original, pre-specified analysis that was erroneously not transferred into the study protocol, uncertainty remains due to the fact that the primary analysis as pre-specified in the protocol failed.
- A large and imbalanced proportion of patients (more in the glutamine than in the placebo arm) discontinued the study and were not followed further (i.e., crises occurring after their discontinuation have not been counted). Consequently, the impact of missing data imputation is large, which increases the uncertainty of the estimation of the treatment effect.

- In analyses using different imputation schemes, the differences in SCC rate were consistently small and did not reach statistical significance.
- Efficacy analyses making use of the investigator-adjudicated dataset yield even worse results than the primary analyses relying on CAC adjudication.
- There is no strong support from secondary endpoints and lack of consistency between subgroups.

In addition, important limitations apply to the Phase 2 study submitted, limiting its supportive value.

Due to the above stated weaknesses of the data presented, the current application is not considered to fulfil the requirements for establishing efficacy.

2.6. Clinical safety

Safety data was mainly presented in a pooled analysis supplementing the safety data of the separate two randomized, double blind, placebo controlled studies (Study GLUSCC09-01 and Study 10478). Limited supportive data from four exploratory studies is available (43 patients). Additional literature concerning the use of glutamine in SCD was presented to support the generated safety data.

Post-marketing data over 10 years is available for the treatment of short bowel syndrome showing a benign safety profile (NutreStore, since 2004 in the US).

Glutamine has further been recently approved in the US for the treatment of sickle cell disease (Endari, since 2017). The Applicant provided five quarterly Periodic Adverse Drug Reaction Reports (PADER) for Endari (data covering the time from October 2017 to December 2018).

Glutamine is also a parenteral nutrition substrate and was licensed as such in many de-centralised and national procedures.

The data provided by the Applicant for PK/PD and non-clinical aspects did not raise any specific safety concerns.

Patient exposure

In total, 298 patients received at least one dose of study medication in the two main studies. Of 187 patients receiving glutamine, 109 patients were exposed for at least 48 weeks (58.3%).

Table 3. Summary of Drug Exposure (Safety Population)

Parameter/Category	Glutamine N = 187 n (%)	Placebo N = 111 n (%)	Total N = 298 n (%)
Duration of exposure (days)[a]			
N	187	111	298
Mean (SD)	268.9 (126.92)	283.3 (121.63)	274.3 (124.96)
Median	350.0	353.0	351.0
Min, max	1, 406	1, 395	1, 406
Patients with exposure, n (%)			
≥ 1 day	187 (100.0)	111 (100.0)	298 (100.0)
≥ 1 week	180 (96.3)	107 (96.4)	287 (96.3)
≥ 2 weeks	179 (95.7)	105 (94.6)	284 (95.3)
≥ 4 weeks	175 (93.6)	104 (93.7)	279 (93.6)
≥ 8 weeks	166 (88.8)	101 (91.0)	267 (89.6)
≥ 12 weeks	161 (86.1)	98 (88.3)	259 (86.9)
≥ 16 weeks	153 (81.8)	93 (83.8)	246 (82.6)
≥ 20 weeks	145 (77.5)	91 (82.0)	236 (79.2)
≥ 24 weeks	136 (72.7)	89 (80.2)	225 (75.5)
≥ 28 weeks	132 (70.6)	85 (76.6)	217 (72.8)
≥ 32 weeks	126 (67.4)	83 (74.8)	209 (70.1)
≥ 36 weeks	124 (66.3)	78 (70.3)	202 (67.8)
≥ 40 weeks	120 (64.2)	76 (68.5)	196 (65.8)
≥ 44 weeks	117 (62.6)	74 (66.7)	191 (64.1)
≥ 48 weeks	109 (58.3)	73 (65.8)	182 (61.1)
≥ 53 weeks	13 (7.0)	10 (9.0)	23 (7.7)
Number of patient-days	50290	31449	81739
Number of patient-years[b]	137.7	86.1	223.8

Studies included: Study 10478 and Study GLUSCC09-01.
Abbreviations: max = maximum, min = minimum, SD = standard deviation
[a] Duration of exposure = last day on study medication – first day on study medication +1, where last day on study medication includes the taper phase.
[b] Number of patient-years = number of patient-days divided by 365.25.
Source: ISS Table 3.

The four supportive studies contribute another 43 patients to the overall safety database. However, in these studies patients were mostly treated for significantly shorter periods of time. Only one of these patients received glutamine for 48 weeks.

In both studies, the applied daily oral doses were 10, 20 or 30g assigned based on body weight, intended to be equivalent to 0.3 g/kg. An additional analysis of AEs by dose cohort (10, 20, or 30 g) was not very meaningful as only TEAE rates (min. one TEAE) were compared but not their quality or the quantity per patient. The TEAE rates (min- one TEAE) are around 100% in all groups and dose levels in the GA and placebo group. For SAEs the rates are around 78% in the 20g and 30g dose groups for GA and placebo. SAEs were recorded less in the 10g group in the GA cohort (58.8%) compared to the placebo cohort (90%). The 10g dose group is considerably smaller than the 20g and 30g groups providing less robust results with this regard.

Analysis of TEAEs and SAEs per body weight categories (<30, 30-65, 65-100 and >100kg) do not reveal any relevant additional findings.

It was noted that the doses used in the studies were rarely equivalent to 0.3g/kg. For example, the dose of 20g - which is intended for patients with 30 – 65 kg - is equivalent to 0.3g/kg for a patient with 65kg but for a patient with 30kg it is equivalent to almost 0.7g/kg. In addition, patients with >100kg would receive less than 0.3g/kg. Whereas inevitable if categories and not individual weight-based dosing is used, an additional discussion of the safety profile in patients that received the highest dose per kg in comparison to lower exposed patients was considered necessary to evaluate possible exposure dependent safety differences.

An analysis of TEAEs and SAEs per g/kg dose (≤ 0.2, 0.3, 0.4, 0.5, 0.6 and ≥ 0.7 g/kg) for both treatment arms did not reveal suspicious trends (see ARs). While the proportion of patients experiencing serious adverse events is higher in the high dose groups ≥ 6 g/kg compared to overall study population, the number of events was balanced between the glutamine and the placebo arms.

Demographics and Baseline Characteristics

The treatment arms seem well balanced in respect to the presented characteristics (age, sex, race, diagnosis) except for Hydroxyurea use at baseline which was slightly more common in the glutamine group.

Table 4. Patient Demographics and Baseline Characteristics (Safety Population)

Parameter/Category	Glutamine N = 187 n (%)	Placebo N = 111 n (%)	Total N = 298 n (%)
Age (years)			
Mean (SD)	23.7 (12.27)	23.1 (12.06)	23.5 (12.18)
Median	22.0	21.0	22.0
Min, max	5, 58	5, 58	5, 58
Age group, n (%)			
5 to 12 years	35 (18.7)	19 (17.1)	54 (18.1)
13 to 18 years	45 (24.1)	29 (26.1)	74 (24.8)
≤ 18 years	80 (42.8)	48 (43.2)	128 (43.0)
> 18 years	107 (57.2)	63 (56.8)	170 (57.0)
Sex, n (%)			
Male	84 (44.9)	53 (47.7)	137 (46.0)
Female	103 (55.1)	58 (52.3)	161 (54.0)
Race, n (%)			
Black or African American	182 (97.3)	107 (96.4)	289 (97.0)
Other	5 (2.7)	4 (3.6)	9 (3.0)
Diagnosis, n (%)			
Sickle cell anaemia	169 (90.4)	99 (89.2)	268 (89.9)
Sickle β^0-thalassaemia	16 (8.6)	12 (10.8)	28 (9.4)
Other[a]	2 (1.1)	0	2 (0.7)
Hydroxycarbamide use at baseline, n (%)			
Yes	124 (66.3)	65 (58.6)	189 (63.4)
No	63 (33.7)	46 (41.4)	109 (36.6)

Studies included: Study 10478 and Study GLUSCC09-01.
Abbreviations: max = maximum, min = minimum, SD = standard deviation
[a] 'Other' includes sickle cell trait (Hgb SC) and sickle β^+-thalassaemia.
Source: ISS Table 2.

Ethnicities:

The majority of patients (97%) are Black or African American. Between treatment groups, these percentages are comparable. There are only 9 patients representing other ethnicities (glutamine: 5, placebo: 4). A similar distribution has been seen in the supportive studies (5 of 43 patients are other than Black/African American).

Diagnosis:

About 90% of the patients were diagnosed with sickle cell anaemia and 10% (28 patients) with sickle β0-thalassaemia. This ratio also corresponds with the estimated population in Europe (Hickman *et al* 1999). Patients with different diagnoses are well balanced between treatment groups. The small number of patients with β0-thalassaemia impairs comparison of glutamine performance in different underlying conditions.

Various forms of SCD exists and differ in disease severity, symptoms and potential outcome. In the GLUSCC09-01 clinical trial, the Applicant focused on the two most severe and most common genetic variants of sickle cell disease, namely sickle cell anaemia and β0 thalassemia.

Age groups:

Sickle cell disease is a heritable disease which usually manifests for the first time with symptoms and deformed erythrocytes in infants at the age of about 6 months when foetal haemoglobin is replaced by HbS. However, SCD can be diagnosed earlier via newborn screening or even genetic testing during pregnancy, which has been an established measure in the US and several European counties (see review in Colombatti *et al* 2016). Although survival in children has improved over the last 20 years, early treatment initiation is essential to prevent organ dysfunction (Quinn *et al* 2010, Colombatti *et al* 2016). The included paediatric population consists of 128 patients aged ≤ 18 years: 80 in the glutamine and 48 in the placebo treatment group. 35 patients between 5 and 12 years and 45 patients between 13 and 18 years received glutamine.

Concomitant medications:

Overall, the classes of medications are well balanced between groups, but also some sparing of certain concomitant medications could be observed in patients treated with glutamine, which could be interpreted as beneficial effect.

Slightly more patients in the glutamine group used HU at baseline compared to patients in the placebo group (62.6% vs 55.0%). The Applicant explained that in study 10478 the classification of HU use at baseline was based on an incorrect question and corrected the values for HU use at baseline to 117 (62.6%) for the glutamine group and 61 (55.0%) for placebo. Hydroxyurea use at baseline was used as stratification factor only in the phase III study GLUSCC01-09.

According to the study protocols, patients were supposed to continue their usual therapy. Only three patients stopped the HU treatment while on glutamine. Reasons for discontinuations were not recorded but the recorded AEs around the time of discontinuation of HU do not give reason for concerns.

Adverse events

Adverse events were monitored throughout the course of both studies via monthly screening visits including adverse event description, onset date, stop date, outcome, frequency, severity, and relationship to study medication, action taken regarding study medication, concomitant treatment, and seriousness. Between visits patients recorded all events in a patient daily diary card, capturing dose interruptions, medications, medical facility visits, and adverse events. The methods applied for monitoring of AEs are considered adequate.

The integrated safety analysis focusses on treatment-emergent adverse events (TEAEs) and drug related events, which is considered acceptable. A TEAE was defined as any AE with an onset date on or after the date of the first dose of study drug through 30 days after the last dose of study drug. An AE was defined as any untoward medical occurrence in a clinical investigation in which a patient was administered a pharmaceutical/biological product, regardless of the relationship of the medical occurrence to the pharmaceutical/biological product.

The rates of TEAEs are high: 96% (glutamine) and 97% (placebo) of patients reported at least 1 TEAE, but also well balanced between the treatment and placebo groups. Drug related events occurred considerably less frequent (glutamine: 19%, placebo: 14%).

The most common TEAEs were sickle cell crisis and acute chest syndrome, which are symptoms associated with the disease.

The analysis of adverse events is based on the number of patients experiencing at least one event in a certain category, and the corresponding crude incidence rate is calculated by dividing this by the total number of subjects in each treatment arm. An overview was provided of how many TEAEs occurred in total and how many patients experienced them. An additional overview about how the AEs were distributed between patients showed that many patients reported more than one AE during the study period (most reported between 1-20 events) and that there is no noteworthy difference in the recorded number of TEAEs per patient recorded between the glutamine and the placebo groups. Furthermore, a balanced distribution of AEs during the weeks/months of study duration was observed with a small decrease of AEs towards the end (where the patient population likely comprises patients that tolerate treatment well). While early after study initiation the highest AE rates were recorded, incidence rates were balanced between the Glutamine and the placebo group throughout the study duration.

Tables picturing adverse events adjusted for exposure time reveal that the overall incidence rates of TEAEs per 100 patient-years were comparable between the Glutamine and placebo group, although slightly higher for glutamine: 130.7 TEAEs per 100 patient-years for glutamine and 125.4 for placebo in both studies. Adjusted incidence rates for drug related events in the safety population showed a higher rate for patients taking glutamine. Also, the corrected rate of patients who discontinued due to AEs was higher compared to Placebo in the ISS (glutamine 25.42 and placebo 17.42). This effect was primarily observed in the phase III study GLUSCC09-01.

Patients who withdrew from treatment were relatively balanced between the Glutamine and the Placebo group (40 out of 111 in Placebo (36.0%) and 72 out of 187 in the Glutamine group (38.5%)). The rate of patients experiencing TEAEs treatment within 2 weeks before up until 1 week after the last day of study medication was quite similar in the Glutamine- and placebo group, a bit lower in the Glutamine group though (Glutamine 38.9%; placebo 45.0%). The rate of patients with TEAEs in the two weeks before discontinuations is lower for glutamine (Glutamine 31.9%; placebo 40.0%). However on the last day of study medication the rate is higher in the glutamine group (Glutamine 9.7%; placebo 2.5%).

Supportive studies

Safety data came also from the fourlegacy studies, where there were no peculiarities concerning AE rates, quality or distribution. No information on the manner or systematic way of collecting safety data is available from these sources.

Common Adverse Events

Some imbalances in individual TEAEs rates of common TEAEs have been noted between glutamine and placebo. Xyndari performs better compared to placebo for the TEAEs pyrexia (17.1% glutamine, 27.9% placebo), acute chest syndrome (10.2% glutamine, 21.6% placebo), and rash (1.6% glutamine, 10.8% placebo).

Other common TEAEs are constipation, nausea, headache, cough, back and chest pain etc. Glutamine seems to cause slightly higher rates of these events when compared to a placebo control group (see section on drug related AEs below).

Ammonia is a main metabolite of GA and its kinetics have not been clarified. Increased ammonia level cannot be ruled out in the SCD population.

Table 6. Summary of TEAEs, Occurring in ≥ 10% of Glutamine-treated Patients, by PT (Safety Population)

PT	Glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 TEAE	180 (96.3)	108 (97.3)
Sickle cell anaemia with crisis	152 (81.3)	97 (87.4)
Constipation	40 (21.4)	20 (18.0)
Nausea	36 (19.3)	16 (14.4)
Headache	34 (18.2)	17 (15.3)
Pyrexia	32 (17.1)	31 (27.9)
Cough	29 (15.5)	15 (13.5)
Pain in extremity	25 (13.4)	8 (7.2)
Upper respiratory tract infection	25 (13.4)	20 (18.0)
Back pain	23 (12.3)	6 (5.4)
Chest pain	23 (12.3)	9 (8.1)
Vomiting	23 (12.3)	14 (12.6)
Arthralgia	22 (11.8)	15 (13.5)
Abdominal pain	19 (10.2)	10 (9.0)
Abdominal pain upper	19 (10.2)	8 (7.2)
Acute chest syndrome	19 (10.2)	24 (21.6)

Studies included: Study 10478 and Study GLUSCC09-01.
AEs are counted only once per patient within the MedDRA category.
PTs are sorted by descending frequency in the glutamine group.
Abbreviations: PT = preferred term; TEAE = treatment-emergent adverse event.
Source: ISS Table 4.3.

Drug-related Treatment Emerged AEs

The definition of 'drug related AEs' seems sufficiently conservative and is considered acceptable. It is noted, however, that the identification of drug related adverse events remains highly subjective and that therefore it is not always a reliable parameter.

Nevertheless, the rates were low and rather balanced across treatment groups, with slightly higher rates for constipation, abdominal pain, nausea and diarrhoea in the glutamine group. In total 35 patients (18.7%) receiving glutamine experienced drug related AEs compared to 13.5% for placebo.

Table 7. Summary of Drug-related TEAEs in ≥ 1% of Glutamine-treated Patients, by PT (Safety Population)

PT	Glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 drug-related TEAE	35 (18.7)	15 (13.5)
Constipation	14 (7.5)	5 (4.5)
Abdominal pain upper	5 (2.7)	1 (0.9)
Nausea	5 (2.7)	1 (0.9)
Abdominal pain	4 (2.1)	4 (3.6)
Diarrhoea	3 (1.6)	1 (0.9)
Vomiting	3 (1.6)	3 (2.7)
Hypersplenism	2 (1.1)	0
Increased appetite	2 (1.1)	0
Pruritus	2 (1.1)	1 (0.9)
Sickle cell anaemia with crisis	2 (1.1)	1 (0.9)

Studies included: Study 10478 and Study GLUSCC09-01.
Drug-related TEAEs are those with a relationship to study drug reported as "possible", "probable", "definite", or missing.
AEs are counted only once per patient within the MedDRA category.
PTs are sorted by descending frequency in the glutamine group.
Abbreviations: PT = preferred term; TEAE = treatment-emergent adverse event.
Source: ISS Table 4.4.

TEAEs by Demographic subgroups

Gender

Women and men reported similar rates of TEAEs between the treatment arms: lower rates for SCC, ACS, Pyrexia and Infections and Infestations while receiving glutamine compared to placebo and higher rates for pain related events (e.g. abdominal pain, back pain), gastrointestinal disorders (e.g. constipation, nausea, diarrhoea), headache, fatigue and TEAEs related to the spleen.

It was noted, that rates for tachycardia were slightly higher in men than in women compared between glutamine and placebo.

Within the glutamine group female and male patients reported similar rates of TEAEs, except for nausea, which was significantly more frequently reported by women (female: 27.2% glutamine, 13.8% placebo; male: 9.5% glutamine, 15.1% placebo). From the provided data it could not be derived whether these events were experienced rather at the beginning of GA treatment and whether they were of transient nature. Most of the respective events were considered mild or moderate AEs. An underlying cause for the gender imbalance remains unknown, but could be attributed to a higher baseline risk for nausea in female patients. However, this is not supported by results from the placebo group.

Age

The Applicant provided TEAE data grouped by different age thresholds: The first was called group 1 and consists of three age categories: 5-12 years, 13-18 years and >18 years. The second - called group 2 – only distinguishes between 5-18 year and >18 year old patients. These classifications were not further explained, however, it seems they have been prospectively defined.

Comparing glutamine and placebo arms, some differences in TEAE rates were observed between age groups:

(i) It was noted, that the rate of SSC is higher in 13-18 year old patients receiving glutamine compared to placebo. The other age groups reported lower rates of SCC for glutamine.

(ii) Patients >18 years of age receiving glutamine reported slightly higher rates of cardiac disorders than for placebo (14.0% vs 9.5%).

(iii) When comparing adults with children and adolescent patients, some TEAEs were reported by more patients ≤ 18 years compared to adults: Pain in extremities were reported in 22.5% of paediatric patients receiving glutamine compared to 8.3% for placebo, back pain was observed in 21.3% of children receiving glutamine and in 4.2% receiving placebo. These dissimilarities were not observed in adult patients.

Race

A slightly higher percentage of other ethnicities reported TEAEs compared to Black or African American patients.

Diagnosis

No differences in TEAE rates have been observed between patients with different diagnoses (sickle cell anaemia or sickle β0-thalassaemia).

Serious adverse events and deaths

Table 9. Summary of SAEs Occurring in ≥ 2% of Glutamine-treated Patients, by SOC and PT (Safety Population)

SOC PT	Glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 SAE	141 (75.4)	89 (80.2)
Blood and lymphatic system disorders	129 (69.0)	80 (72.1)
Sickle cell anaemia with crisis	124 (66.3)	80 (72.1)
Acute chest syndrome	13 (7.0)	21 (18.9)
General disorders and administration site conditions	16 (8.6)	8 (7.2)
Chest pain	5 (2.7)	2 (1.8)
Pyrexia	5 (2.7)	4 (3.6)
Infections and infestations	18 (9.6)	19 (17.1)
Pneumonia	9 (4.8)	10 (9.0)
Pregnancy, puerperium and perinatal conditions	4 (2.1)	3 (2.7)
Pregnancy	4 (2.1)	3 (2.7)
Respiratory, thoracic and mediastinal disorders	10 (5.3)	8 (7.2)
Asthma	4 (2.1)	3 (2.7)

Studies included: Study 10478 and Study GLUSCC09-01.
AEs are counted only once per patient within the MedDRA category.
Abbreviations: PT = preferred term; SAE = serious adverse event; SOC = system organ class.
Source: ISS Table 4.7.

Rates of serious adverse events are balanced between the groups. They are rather high (glutamine: 75.4%; placebo 80.2%) as could be expected considering the severity of the underlying condition.

The most common SAEs are sickle cell crisis (66.3% vs 72.1%) and acute chest syndrome (7.0% vs 18.9%).

The rate of infections and infestations is lower in the glutamine group (glutamine: 9.6%; placebo 17.1%), especially for pneumonia (glutamine: 4.8%; placebo: 9.0%). Unfortunately, no data concerning the vaccination status of the patients was collected during the studies. Vaccination against encapsulated bacteria

is recommended for SCD-patients in the US according to CDC (https://www.cdc.gov/ncbddd/sicklecell/healthyliving-prevent-infection.html), as all homozygous SCD patients are considered to be at high risk of pneumococcal, haemophilus B and meningococcal infections. All participating centres were located in the US.

Rates of SAEs related to study drug are very low and smoothly balanced across groups. Only three patients in each group reported SAEs that were judged to be related to the study drug. In the glutamine group those SAEs were: hypersplenism, sickle cell anaemia with crisis, abdominal pain, and chest pain. For placebo: sickle cell anaemia with crisis, abdominal pain, constipation, and back pain.

Hydroxyurea use

SAEs rates were higher in patients receiving Hydroxyurea at baseline in both, the glutamine and the placebo groups (glutamine: 82.9% vs 62.9%; placebo: 86.9% vs 72.0%). It has however also been noticed that different thrombosis events occurred in three patients in the glutamine group and none in placebo. All three affected patients were grouped as taking Hydroxyurea at baseline. It was further noted, that three of four patients that died during the study were in the glutamine group and concomitantly took Hydroxyurea. (The fourth was also in the glutamine group but did not take HU for 10 years prior to study participation).

Higher rates of SAEs were also observed for SCC and ACS for patients receiving HU and glutamine as compared to glutamine alone (SCC: 72.6% vs 54.0%; ACS 8.9% vs 3.2%). Other AEs are quite balanced between groups. Different non-serious pain events have also been reported with higher rates for the combination of HU and glutamine (chest pain: 14.5% vs 7.9%; pain in extremities: 14.5% vs 11.1%; Arthralgia: 13.7% vs 7.9%).

The rates of all other SAEs were very low and similar between patients irrespective of Hydroxyurea use at baseline.

Deaths

Four deaths have been reported in the glutamine group and none for placebo (three in study GLUSCC09-01, one in study 10478, and none in the legacy studies). Three deaths were due to TEAEs and one was not considered treatment-emergent. All four were judged to be not related to the study drug.

According to the narratives provided by the Applicant, a 37 year old woman died due to anaemia, renal and respiratory failure. The other three patients died of cardiac arrest during sickle cell crises (a 46 year old woman, a 45 year old man and a 10 year old boy). The 10 year old boy died 120 days after the last dose of glutamine was taken and his death was therefore not considered as treatment related.

It is noted that in SCD life expectancy is decreased to 42 - 53 years for men and 48 - 58 years for women (Platt et al 1994, Wierenga et al 2001, Lanzkron et al 2013). Events related to vaso-occlusive crises are the most common cause of death for sickle cell patients. However, the rate of those events is supposed to be lowered by the glutamine treatment.

The two older patients who died of cardiac arrest during sickle cell crises (both in the Glutamine group) during the treatment phase of the studies were multimorbid and had cardiovascular risk factors (one was diagnosed with systolic ejection murmur, the other had a history of atrial fibrillation, hypertension, pulmonary hypertension, and renal failure). These two patients were 45 and 46 years old, which is in the range or close to the lower limit of the range of life expectancy for patients with SCD.

All four deaths occurred in patients who took Hydroxyurea concomitantly with glutamine. It was later clarified that this was the case for three of them only.

Laboratory findings

Laboratory screenings include complete blood count and reticulocyte count measured every 4 weeks. Serum chemistry and hepatic panel were collected at the initial screening and the end of the study. The measured parameters are overall acceptable. However, to assess potential effects of metabolites, measurement of glutamate and ammonia levels would have been beneficial.

Four patients experienced significant changes in blood chemistry parameters. These changes did not concern the same parameter and did not show a consistent trend over all four patients.

A slightly greater mean increase was observed in the glutamine group compared with placebo for systolic blood pressure (1.84 mmHg vs -0.14 mmHg) and pulse rate (2.90 beats per minute [bpm] vs 1.11 bpm). It is agreed that the reported observations over the course of 48 weeks are unlikely to be clinically relevant.

Otherwise, no indication of relevant influence of glutamine on laboratory parameters (haematology and serum chemistry parameters) and vital signs was detected. No clinically notable differences from baseline between glutamine treatment and placebo were observed for blood and platelet counts. However, some differences might have been expected for erythrocytes, as a reduction in sickling could be expected to result in higher counts of erythrocytes. Also serum chemistry parameters did not show any clinically notable differences between treatment groups or between baseline and end of study.

Safety in special populations

Renal and hepatic Insufficiency

Patients with severe renal or hepatic insufficiency were not studied and were specifically excluded from studies 10478 and GLUSCC09-01. However, patients with minor renal and hepatic abnormalities at baseline were included and monitored. The available data do not indicate any differences concerning AEs for those patients between the glutamine and the placebo group. Nonetheless, no robust conclusions can be made due to their limited number and due to the only minor abnormalities.

Glutamine is metabolised to glutamate and ammonia in liver and kidneys, both can cause unfavourable neurological effects. Published studies (reviewed in Garlick 2001) indicate that blood level of both do not increase during the treatment with glutamine. However, those studies were only conducted over a considerably shorter period of time and in patients without organ damage. It has been shown, that glutamine metabolism is altered and blood ammonia levels are increased in patients with acute liver failure and liver cirrhosis (Oppong *et al* 1997, Clemmesen *et al* 2000, Ditisheim *et al* 2011). An increased ammonia level is associated with a variety of detrimental effects causing, among others, neurological symptoms.

The presented literature overview indicates concerns for patients with hepatic and renal failure (significantly increased 28-day mortality in critically ill ICU patients with renal dysfunction). As the metabolism of glutamine involves both, kidney and liver and damage to these organs is not uncommon in SCD patients, a risk exists that an altered metabolism may lead to pharmacologically relevant levels of glutamine metabolites (e.g. glutamate, ammonia).

Paediatric Population

See above under the heading 'TEAEs by Demographic subgroups'.

Elderly population

No data is available for glutamine use in the elderly population >65 years of age. The oldest patient included in the safety dataset was 58 years old. However, the definition of an elderly population (>65 years) is not particularly relevant for patients with SDC as life expectancy is decreased to 42 - 53 years for men and 48 - 58 years for women for patients with SCD (Platt *et al* 1994, Wierenga *et al* 2001, Lanzkron *et al* 2013). It is stated in the product information that dose selection for elderly patients should be carefully adjusted in view of a greater frequency of decreased hepatic, renal, or cardiac function, and concomitant disease or other drug therapy.

Use in Pregnancy and Lactation

No reproduction studies have been performed with glutamine. Although studied in gilts and not in humans, a publication in 2012 showed that glutamine supplementation increases the free glutamine in milk during lactation. This indicates a probable effect of glutamine supplementation for nursing women.

Overdose

No data for humans are presented. However, single lethal doses of glutamine have been discovered in mice, rats and rabbits. These finding can be converted into a potentially lowest lethal dose in human of 1.2g/kg via allometric conversion. This is approximately 2-fold more than the maximum dose received by a patient in the presented studies (0.6 g/kg). Additionally, much higher doses of >200g/kg for total protein have been shown to be toxic in human (reviewed in Garlick 2001).

Drug Abuse

Glutamine is widely used as nutrition supplement and as such freely available also in similar doses as recommended for SCD patients.

Withdrawal and Rebound

Any effects of withdrawal are unlikely to have been detected, because both studies included a tapering phase for each patient to prevent the sudden onset of sickle cell crises. However, several studies, mainly in healthy athletes, indicate that a higher rate of infections can occur at sudden withdrawal after long term treatment with glutamine. As patients with sickle cell anemia are considered to have a more compromised immune response, this could be a potential risk. In addition, tapering could be necessary to prevent sudden onset of sickle cell crises.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Ability

Effects on the ability to drive or operate machinery are generally not expected based on the presented safety profile.

Immunological events

No data was presented on immunological events with glutamine. The risk of possible immunological events can be considered low as glutamine is an endogenous substance and naturally highly abundant in human blood.

Safety related to drug-drug interactions and other interactions

Concomitant medication is very common in SCD patients and some agents could possibly influence the treatment performance (safety and efficacy) of glutamine. However, no interaction studies have been performed and only limited non-clinical data are available with this regard.

Furthermore, no studies were presented that examined potential interactions between glutamine and drugs commonly used by patients with SCD, like Hydroxyurea and opioids. No CYP enzymes are involved in glutamine metabolism.

Hydroxyurea is a common concomitant medication. The known adverse events of HU involving kidney and liver might have an influence on the elimination of glutamine and its metabolite ammonia.

Furthermore, several recent publications showed that glutamine decreases blood glucose level, which could result in a reduced need for insulin in diabetes patients (type 1 and 2) (Samocha-Bonet *et al* 2011, Badole *et al* 2013, Samocha-Bonet *et al* 2014, Samocha-Bonet *et al* 2015, Torres-Santiago *et al* 2016, Wang *et al* 2018).

Discontinuation due to adverse events

The number of patients who discontinued in total is similar between treatment arms (glutamine: 38.5%, placebo 36.0%) in the ISS. However, the discontinuation rate differs between studies and treatment arms. In study GLUSCC09-01 35.8% of patients discontinued in the glutamine group and 24.4% in the placebo group. In the smaller study, study 10478, 51% discontinued in the glutamine group and 63% in the placebo arm. The reasons for discontinuation were similar between treatment groups and there seems to be no influence of demographic factors on discontinuation in general or especially on discontinuations due to AEs. Only six patients discontinued their treatment due to AEs (5 glutamine; 1 placebo). The drug-related TEAEs that led to withdrawal in the glutamine treatment group were hypersplenism, abdominal pain, dyspepsia, and hot flush (1 patient each [0.5%]).

The most common reason for discontinuation was 'consent withdrawn' (glutamine 13.9%; placebo: 12.6%). Other important terms for discontinuations were identified as "other" and "non-compliance". The Applicant provided narratives for patients in the categories "consent withdrawn" and "others". These indicate that 14 patients reported AEs before withdrawing their consent. The Applicant confirmed that those AEs have been included in the safety database. Other patients discontinued for reasons such as 'too much medication' or 'too difficult to stick to the schedule', which might be better referred to as 'non-compliance' than 'consent withdrawn'. Although requested, the Applicant did not discuss the underlying reasons for "non-compliance".

No pattern can be found when looking at the underlying reason for 'other reasons' for discontinuation. Five of the 26 patients were excluded due to violations of inclusion criteria (3 in study 10478 and 2 in study GLUSCC09-01), questioning diligence of recruitment methods.

It has also been noted that while rates of discontinuations overall are equal for 5-12 year old (glutamine: 29.4%, placebo: 29.4%) and discontinuations in adults are quite balanced between glutamine and placebo (35.5% vs 28.6%), the difference in 13-18 year old patients is higher (glutamine: 41.5%, placebo: 15.4%). The collected reasons are however sparse but some indicate 'no apparent benefit' and that the treatment is often seen as 'nuisance' to patients and parents.

Table 2. Summary of Patient Disposition (Safety Population)

Parameter/Category	Glutamine N = 187 n (%)	Placebo N = 111 n (%)	Total N = 298 n (%)
Completed the study[a]	115 (61.5)	71 (64.0)	186 (62.4)
Discontinued from study[a]	72 (38.5)	40 (36.0)	112 (37.6)
Reasons for discontinuation[a]			
Consent withdrawn	26 (13.9)	14 (12.6)	40 (13.4)
Noncompliance	17 (9.1)	10 (9.0)	27 (9.1)
Lost to follow-up	6 (3.2)	4 (3.6)	10 (3.4)
AEs	5 (2.7)	1 (0.9)	6 (2.0)
Death	3 (1.6)	0	3 (1.0)
Other	15 (8.0)	11 (9.9)	26 (8.7)

Studies included: Study 10478 and Study GLUSCC09-01.
Abbreviation: AE = adverse event.
[a] Percent based on total N in group.
Source: ISS Table 1.

Table 10. TEAEs That Led to Withdrawal in 1 or More Patient, by SOC and PT (Safety Population)

SOC PT	Glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 TEAE that led to withdrawal	5 (2.7)	1 (0.9)
Blood and lymphatic system disorders	1 (0.5)	0
Hypersplenism	1 (0.5)	0
Gastrointestinal disorders	2 (1.1)	0
Abdominal pain	1 (0.5)	0
Dyspepsia	1 (0.5)	0
Nervous system disorders	1 (0.5)	0
Burning sensation	1 (0.5)	0
Pregnancy, puerperium and perinatal conditions	1 (0.5)	1 (0.9)
Pregnancy	1 (0.5)	1 (0.9)
Vascular disorders	1 (0.5)	0
Hot flush	1 (0.5)	0

Studies included: Study 10478 and Study GLUSCC09-01.
AEs are counted only once per patient within the MedDRA category.
Abbreviations: PT = preferred term; SOC = system organ class; TEAE = treatment-emergent adverse event.
Source: ISS Table 4.9.

Literature

The Applicant provided an overview of identified literature concerning the use of glutamine.

Many of the selected 84 references concern glutamine use in the context of parenteral nutrition supplementation over a limited time frame. A wide range of patients with different underlying diseases is

covered. When AEs are reported, the quality of these AEs is mostly in line with what was seen in the Xyndari program, being predominantly gastrointestinal effects.

Some further literature including different critically ill patient populations, not SCD patients, in whom Glutamine was used as nutrition supplementation with different routes of administration (iv and oral) is also included. In the majority of the presented conditions (e.g. trauma, burns, chemotherapy etc.) no negative effects were (clearly) attributed to glutamine use. However, the additional literature does not include patients with renal or hepatic impairment, dysfunction or failure, which are of interest also according to information from other sources discussed in other parts of this report (e.g. PD). Respective patients are only included in the study published by Heyland et al, where the 28-day mortality was increased significantly for patients with renal dysfunction receiving glutamine or antioxidant or both, indicating an increased risk for patients with renal impairment.

A discussion based on literature concerning possible safety concerns relating to the use of high doses of glutamine and glutamine dependent (pre-existing) tumours did not indicate an increased risk.

Post marketing experience

Glutamine (NutreStore) is approved for SBS. Annual periodic safety update reports (PSURs) have been submitted to the US Food and Drug Administration (FDA) and a summary of safety data provided in the PSURs covering the period from 11 Jun 2016 to 10 Jun 2018 has been included in this application. Safety data reported in the periodic adverse drug experience reports, since the launch of Endari in the United States was also provided.

2.6.1. Discussion on clinical safety

Glutamine is a well characterised amino acid. It is usually synthesised in sufficient amount in the human body and is one of the most abundant amino acids. Under stress, additional intake of glutamine can be required. It is present in a variety of protein-rich food and usually about 6g are consumed on average per day with a normal diet. Furthermore, glutamine is widely used as nutrition supplement with daily doses up to 30-40g and as such used e.g. to support protein synthesis and promote muscle growth. The proposed daily doses for SCD patients are comparable to how nutrition supplements are used and about two- to tenfold the amount consumed with normal diet.

For this full application, safety data was mainly presented in a pooled analysis of two randomized, double blind, placebo controlled studies (Study 10478, Phase II and GLUSCC09-01, Phase III). Limited supportive data from four exploratory studies is available (43 patients).

Exposure

In total, 298 patients received at least one dose of study medication in the two main studies. Of 187 patients receiving glutamine 109 patients were exposed for at least 48 weeks (58.3%), 146 patients for 24 weeks (including the supportive studies). This is considered borderline sufficient to allow adequate assessment of the drug's (safety) performance according to the requirement of ICH E1, taking into account the orphan situation, the information from post-marketing data from other authorisations and the relatively large bibliographical database on the matter.

It was noted that 7.7% of subjects were exposed to the drug for 53 weeks in both studies instead of 48 plus tapering as expected and as specified in the provided study protocols. The Applicant clarified that this can be

attributed to the variation of visit schedules. Visits were scheduled every 4 weeks ± 7 days, where a 5-week supply of study drug was dispensed. The Applicant explained that over a study of this duration it is not unusual for some subjects to have visits that occurred later than the scheduled visit day but still within the specified product timeline, finally resulting in the observed accumulative effect. There does not appear to be a clear preponderance of this issue in a particular group/study arm.

Follow-up/exposure durations differ between study arms in the phase III study, which needs to be considered when comparing incidence rates between Xyndari and placebo arms. Shorter exposure/monitoring times could result in an absolute smaller number of AEs reported in this arm, which could shift the relation in favour of Xyndari. Adverse events adjusted for exposure reveal that the incidence rates of TEAEs per 100 patient-years were comparable between the Glutamine and placebo group, however. Adjusted incidence rates for the safety population showed a higher rate of drug related events for glutamine and a higher rate of patients who discontinued due to AEs compared to Placebo in the ISS (glutamine 25.42 and placebo 17.42) (driven by GLUSCC09-01).

The Applicant reports that one patient was enrolled twice in study 10478 (04 May 2006 to 14 Jun 2006 and 07 Sep 2006 to 06 Sep 2007) and received glutamine both times. The safety data is included for both enrolments. The incidence raises some concerns about the integrity of the study in total. Also other issues were discovered for study 10478 during the assessment, e.g. misclassification of HU-use at baseline (see "concomitant medication") or violations of inclusion criteria (see "discontinuations").

The proposed daily doses for Xyndari are 10, 20 and 30g (assigned based on body weight, intended to be equivalent to 0.3 g/kg). Nevertheless, in the clinical studies the proposed dosage groups introduce different per kg doses from < 0.3g/kg up to 0.7 g/kg. This can be seen as an advantage for the evaluation of safety as data from higher per kg doses are available for assessment. A dose dependency of AEs has not been observed.

Population

The majority of patients (97%) are Black or African American. Between treatment groups, these percentages are comparable. There are only 9 patients representing other ethnicities (glutamine: 5, placebo: 4). A similar distribution has been seen in the supportive studies (5 of 43 patients are other than Black/African American). The prevalence of SCD worldwide in ethnicities other than Black/African American is rather low. Therefore, although this ethnicity proportion does not correspond to the overall European population, it does reflect the population suffering from sickle cell disease in Europe (Hickman *et al* 1999, Gulbis *et al* 2006, Modell *et al* 2007, Roberts *et al* 2007, Colombatti *et al* 2016). Conclusions on safety made based on this population are therefore considered relevant for a (possible) approval in the European Union.

Various forms of SCD exists differing in disease severity, symptoms and potential outcome. In the GLUSCC09-01 clinical trial, the Applicant focused on the two most severe and most common genetic variants of sickle cell disease, namely sickle cell anaemia and β0-thalassemia. About 90% of the patients were diagnosed with sickle cell anaemia and 10% (28 patients) with sickle β0-thalassaemia. This ratio also corresponds with the estimated population in Europe (Hickman *et al* 1999). Patients with different diagnoses are well balanced between treatment groups.

From an efficacy perspective, as the MoA of Xyndari has not been fully characterised, there is no strong evidence based on which the extrapolation to other genotypes than those evaluated in clinical trials can be justified. The benefit of glutamine treatment of genotypes with a milder form of SCD is currently unclear. Incidences of safety events of interest between more and less severely affected SCD patients cannot be assessed as these data are not available and there were not many patients with 'mild' disease included in the

studies. Extrapolation of available data concerning the safety profile to other disease groups/genotypes/ severities seems defendable, however. Nevertheless, it cannot be entirely excluded that the B/R for Xyndari in patients with less 'severe' disease differs from the population studied.

Concomitant medication is very common in SCD patients and some agents could possibly influence the treatment performance (safety and efficacy) of glutamine. An effect of glutamine on other treatments cannot be ruled out, e.g. recent literature reported glutamine lowers blood glucose level. However, no interaction studies were provided. A recommendation was proposed in section 4.5 of the SmPC that Glutamine has been suspected to cause reduction of blood glucose level and that there could be a need for dose adjustment of diabetes medication in patients receiving medication for the treatment of diabetes Type I and II.

Adverse events

Relevant TEAES observed in the trials were the known glutamine TEAEs; constipation (glutamine: 21.4%; placebo: 18.0%), nausea (glutamine: 19.3%; placebo: 14.4%) and headache (glutamine: 18.2%; placebo: 15.3%) all of which occurred at higher rates in the glutamine group and which were adequately reflected in the SmPC. Rates of serious adverse events are low and balanced between groups.

Four deaths have been reported in the glutamine group (three in study GLUSCC09-01, one in study 10478) and none for placebo. Three of them died of cardiac arrest during sickle cell crises. All four were judged to be not related to the study drug; however, the fact that the deaths occurred only in the glutamine group and none with placebo is striking.

In order to be able to assess the potential connection of glutamine and events like cardiac arrest, predispositions for cardio vascular events and related risks for the deceased patients were further discussed: Balanced rates for cardiac arrest were observed between treatment arms (glutamine: 2 (1.3%), placebo 1 (1.3%, this patient did not die), corrected for exposure time: 1.5 events for glutamine and 1.2 events for placebo per 100 patient-years). They do not indicate an increased risk for cardiac events with glutamine treatment in SCD patients. However, it is still unclear if there could be an increased risk for cardiac events for patients with pre-existing risk factors. The small overall number of cardiac events and related fatalities in the study sample renders speculations of connections to treatment or other root causes difficult. Considering the narratives on these cases, a relation to study drug seems unlikely, but cannot be completely ruled out.

The *concomitant use of Hydroxyurea* (62.6% of patients at baseline) is of special interest for this assessment. Three of four fatalities recorded in the clinical Xyndari studies occurred in patients that took Hydroxyurea concomitantly with glutamine (no one died in the placebo group). In addition, several thrombosis events only occurred in patients receiving both drugs concomitantly. In analogy to the difficulties of causal attribution to Xyndari use, it is unlikely, yet not fully ruled out that concomitant intake of Xyndari and Hydroxyurea had a bearing on these occurrences.

More patients taking both (glutamine and HU) compared to patients taking glutamine alone experienced different pain events (chest pain: 14.5% vs 7.9%; pain in extremities: 14.5% vs 11.1%; Arthralgia: 13.7% vs 7.9%) and SCC (86.3% vs 71.4%), ACS (11.3% vs 7.9%) (see efficacy assessment). An increased risk for adverse events pertaining to pain or to serious thrombotic events for the combination therapy of glutamine with HU cannot be ruled out based on the provided data. As the overall incidence rates are small, the sample sizes studied are small and the underlying condition could trigger similar events, the risk is hard to determine which introduces uncertainty for the B/R. In addition to those differences, the known adverse events of HU involving kidney and liver might have an influence on the elimination of glutamine and its metabolite ammonia. A respective warning in the SmPC was included for renal and hepatic impaired patients. More

information would be needed to assess a possible negative interaction between glutamine and HU use in SCD patients.

There are some indications from the literature that there could be an increased risk for infections after sudden withdrawal from glutamine. In addition, a potential risk of sudden onset of SCC was of interest for the procedure. In the clinical studies, tapering was introduced as a precautionary and safety measure and all patients who finished the studies were tapered. Comparison of infection rates and SCC rates between patients with and without tapering (patients that withdrew early from study and abstained from tapering) from the clinical Xyndari studies are not sufficiently informative and therefore it was proposed to include this information in the SmPC.

The Applicant provided literature data concerning the use of glutamine to complement the generated safety data. A literature search was conducted using a limited amount of search terms (synonyms for glutamine or names of relevant metabolites seem to not have been considered). The quality of the references (conduct of the experiments, reporting and applicability to this procedure) is moderate. A list of articles found and selected during the literature selection process seems missing. The literature search (i) cannot be followed/reproduced and (ii) the major share of the identified articles could not be reviewed which introduces uncertainties.

Many of the selected 84 references concern glutamine use in the context of parenteral nutrition supplementation over a limited time frame, which is not really comparable with the intended use of Xyndari (long-term use). A wide range of patients with different underlying diseases (benign-severe) is covered within the different publications and outcomes are variable but seem mostly supportive of a benign glutamine safety profile. The way safety was monitored is explained fragmentarily only in few of the provided publications. When AEs are reported, the quality of these AEs is mostly in line with what was seen in the Xyndari program, being predominantly gastrointestinal effects. These are covered in the proposed product information.

Some literature data indicate concerns for patients with hepatic and renal failure (significantly increased 28-day mortality in critically ill ICU patients with renal dysfunction, Heyland *et al.,* 2013). Increased ammonia levels have been reported for patients with reduced liver function (Oppong *et al* 1997, Clemmesen *et al* 2000, Ditisheim *et al* 2011). Information on glutamine safety in patients with renal or hepatic impairment is sparse, as they were excluded from the clinical studies. Special care should be taken for respective patient populations and respective recommendations were therefore included in the SmPC. Patients with renal and hepatic insufficiency were regarded as missing information in the RMP.

Post-marketing data showed a benign safety profile with no AEs reported over 10 years for NutreStore (glutamine approved for Short bowel syndrome). NutreStore has further been supplied for a study in patients with burn injuries, where 65 SAEs have been reported since 2006. The reported SAEs seem in accordance with the underlying injuries and complications following burn trauma. One event of "Acute Kidney Injury" has been considered as "possibly related". Respective warnings for use of Xyndari in patients with kidney disease were already included in the SmPC. Post marketing data from SCD patients are available since January 2018 and do not indicate further safety concerns so far.

Assessment of paediatric data on clinical safety

A total of 80 patients aged 5-18 years were included in the glutamine and 48 in the placebo treatment groups in the main studies.

Considerably more pain related events (unbalanced, favouring placebo) were reported by paediatric patients compared to adults (patients < 18 years: pain in extremities: glutamine 22.5%; placebo 8.3% back pain: glutamine 21.3%, placebo 4.2%). The underlying cause for these differences between age cohorts remains unclear. Pain as a reaction to glutamine treatment in children has to be considered as possibly treatment related.

2.6.2. Conclusions on the clinical safety

Data from clinical studies indicate an overall benign safety profile for glutamine and this notion is supported by post-marketing data and literature reports to some extent. The observed imbalance in fatalities disfavouring Xyndari studies cannot be fully explained. A causal relationship with Xyndari appears unlikely but respective conclusions are severely hampered by the very low event count. The same applies to the concomitant use of Xyndari with HU, where 3 of 4 fatalities, more serious thrombosis events and more pain events were seen.

2.7. Risk Management Plan

The CHMP and PRAC, having considered the data submitted in the application were of the opinion that due to the concerns identified with this application, the risk management plan version 0.3 which was submitted in response to the second D180 joint overview PRAC/CHMP updated assessment report, cannot be agreed at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

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

Periodic Safety Update Reports submission requirements

Not applicable

2.9. Product information

Not applicable

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Xyndari (glutamine) is intended for the prevention of sickle cell crises in adults and children 5 years and older suffering from Sickle Cell Disease (SCD).

SCD is a genetic disorder. In patients with SCD haemoglobin is altered causing the deformation of erythrocytes and subsequent vaso-occlusive events and chronic haemolytic anaemia. The main manifestations of SCD include painful crises including chest, back and joints, organ damage and varying degrees of anaemia and related symptoms.

3.1.2. Available therapies and unmet medical need

The management of sickle cell crises (SCC) is generally supportive and focuses on symptomatic treatment with fluids, analgesics, oxygen and RBC transfusion. Blood transfusion is usually combined with iron chelators (medicines used to reduce the high iron levels in the body caused by repeated blood transfusions), which are necessary in patients with long-term anaemias such as sickle cell disease. Hydroxycarbamide (or Hydroxyurea, HU) (SIKLOS) is approved since 2007 for the prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in adults, adolescents and children older than 2 years suffering from symptomatic Sickle Cell Syndrome. In some cases, haematopoietic (blood) stem cell transplantation has been used.

Providing an alternative treatment option besides current preventive standard of care (i.e., hydroxyurea) may accommodate an unmet medical need for patients who cannot tolerate HU and as add-on in patients experiencing crises despite HU treatment.

3.1.3. Main clinical studies

Key design features of the single pivotal trial (GLUSCC09-01) and a supportive phase-II trial (Study 10478) are described below:

Trial ID Number of Centres and Location Study Dates Start-Completion	Type of Study Design, Control Type	Study Objective	Treatment Dose, Route, Regimen	Treatment Duration	No. of Patients by Treatment Randomised / FAS or ITT	Primary Efficacy	Key Secondary Efficacy
Pivotal Study							
Study GLUSCC09-01 US: 31[a] sites 21 Jun 2010 to 19 Dec 2013	Phase 3 randomised, double-blind, placebo-controlled, parallel-group	Efficacy and safety of oral glutamine therapy for patients with sickle cell anaemia or sickle β^0-thalassaemia who were ≥ 5 years old	Glutamine 0.3 g/kg or placebo, oral, bid	48 weeks, followed by 3 weeks of tapering	Glutamine: 152/152 (ITT) Placebo: 78/78 (ITT)	Number of SCCs through Week 48, CMH test, controlling for region and hydroxycarbamide use, treatment difference ITT: median number of SCCs in the glutamine group was 25% less or 1 SCC lower than for placebo (p = 0.0052) Statistically significant differences favouring glutamine for sensitivity analyses by region (p = 0.0067), hydroxycarbamide use (p = 0.0041), and no covariates (p = 0.0039).	The median number of hospitalisations for sickle cell pain was approximately 33% lower or 1 hospitalisation fewer for the glutamine group than for placebo (p = 0.0045). Number of ER visits for sickle cell pain through Week 48 were the same across treatment groups (p = 0.128)
Supportive Study							
Study 10478 US: 4[b] sites 23 Apr 2004 to 29 May 2008	Phase 2 randomised, double-blind, placebo-controlled, parallel-group	Efficacy and safety of oral glutamine therapy for patients with sickle cell anaemia or sickle β^0-thalassaemia who were ≥ 5 years old	Glutamine 0.3 g/kg or placebo, oral, bid	48 weeks, followed by 3 weeks of tapering	Glutamine: 37/33 (FAS) Placebo: 33/29 (FAS)	Number of SCCs through Week 48, CMH test, controlling for centre, treatment difference FAS: Median number of SCCs 4 vs 5 There was a tendency toward fewer SCCs in the glutamine group compared to the placebo group (p = 0.076)	Fewer number of hospitalisations for sickle cell pain (median 1 vs 2; CMH test, p = 0.072), and ER visits for sickle cell pain through Week 48 with glutamine treatment (median 2 vs 3; CMH test, p = 0.129).

bid = twice daily, CMH = Cochran-Mantel-Haenszel, ER = emergency room, FAS = full analysis set, ID = identification, ITT = intent-to-treat, SCCs = sickle cell crises, US = United States, vs = versus.

[a] One site (investigator: Hussein) was closed and another site (investigator: Woods) replaced it; both sites were considered Site 20.

[b] There were initially 5 sites; however, Site 106 was removed from the study for potential investigator misconduct (see Study 10478 Section 9.6).

Source: Study 10478 CSR, Study GLUSCC09-01 CSR, ISE Table 1.1, ISE Table 1.1.1, and ISE Table 1.6.1.

3.2. Favourable effects

Pivotal study GLUSCC09-01

Primary efficacy endpoint: Sickle Cell Crises through week 48

An analysis using negative binomial regression imputing the mean SCCs of placebo completers or actual SCC count (whichever was higher), shows a rate ratio favouring glutamine after 48 weeks of treatment (point estimate: 0.89, 95.5%CI: 0.75, 1.06), which is not statistically significant (p = 0.193). Analysis using multiple imputation techniques (imputing missing observations based on data from placebo completers) and negative binomial regression shows a rate ratio favouring glutamine after 48 weeks of treatment (point estimate 0.82, 95.5%CI: 0.65, 1.04, p = 0.092) which is not statistically significant. The respective mean values for SCCs are 3.54 vs. 3.97 for glutamine vs. placebo.

In the pre-specified primary analysis (according to study protocol), the difference between treatment arms was not statistically significant either (p=0.063). The median number of sickle cell crisis was 3 vs. 4 crises for the L-glutamine and placebo groups, respectively. The respective mean values were 3.2 vs. 3.9 crises for both groups respectively.

Secondary efficacy endpoints

For analyses using multiple imputation and negative binomial regression, rate of hospitalisations and rate of emergency room (ER) visits per 48 weeks both showed a rate ratio consistent with the primary endpoint and in favour of glutamine (0.82 (95%CI: 0.62, 1.08)) and 0.75 (95%CI: 0.51, 1.10), respectively), neither of which statistically significant.

Additional endpoints

The rate ratio for acute chest syndrome (ACS) favoured L-glutamine (0.41, 95%CI: (0.18, 0.92)).

Cumulative duration of SCCs favoured the glutamine arm (rate ratio of 0.88, 95%CI: (0.63, 1.24)).

Relevant subgroups:

The above stated results apply by and large irrespective of concomitant hydroxyurea use (at baseline), sex, and baseline SCC count. A treatment-by-age interaction was observed in the GLUSCC09-01 study, showing that placebo patients aged 13-18 years experienced fewer SCCs as compared to glutamine treated patients.

Supportive study 10478:

Primary efficacy endpoint: Sickle Cell Crises trough week 48

In an analysis using negative binomial regression with an approach imputing the mean SCCs of placebo completers or actual SCC count (whichever was higher), results indicated a significantly lower rate of SCCs for glutamine than for placebo (rate ratio: 0.56, p = 0.012). Analyses using multiple imputation techniques (imputing missing observations based on data from placebo completers) and negative binomial regression showed a rate ratio of 0.45 after 48 weeks of treatment (95%CI: 0.22, 0.90, p=0.025).

Secondary efficacy endpoints

The results of a negative binomial regression analysis and multiple imputation based on placebo completers of rate of hospitalizations per 48 weeks showed a rate ratio (95%CI) of 0.67 (0.33, 1.35).

3.3. Uncertainties and limitations about favourable effects

From a non-clinical and clinical perspective, significant uncertainty exists with respect to the proposed mode of action which is based on general observations derived from scientific literature and does not involve an elucidation of the underlying mode of action. Data provided on the proposed inhibitory effect of glutamine on the adhesion of sickle RBC are not of sufficient extent and quality to support the proposed pharmacodynamic effect. There is no direct evidentiary link between glutamine supplementation and the stated effect on red blood cells affected by SCD. No patient data have been provided demonstrating a beneficial effect of glutamine on the oxidative damage of sickle RBC (e.g. reduced sickling).

The pharmacology of Xyndari has not been adequately characterised and the presented literature data does not enable a conclusive description of the PK profile. Besides there is a lack of information with regard to the PK profile in the intended target population and no definite statements can be made for special populations of interest.

With regards to the main outcomes from the single pivotal trial and the supportive study, efficacy has not been conclusively demonstrated. Pivotal study GLUSCC09-01 and supportive study 10478 both failed their pre-planned primary analyses.

The initially submitted post-hoc work-up of study results including re-analysis bears the important risk of being data-driven and the Applicant's intent to consider the related 'integrative analysis' as their primary dataset/analysis is not supported.

A high number of patients withdrew from the studies. Furthermore, there is an imbalance between the treatment arms in withdrawal in study GLUSCC09-01: 36% in the glutamine arm compared to 24% in the placebo arm. This questions how missing data is dealt with (data imputation) as well as the choice of statistical method to assess the treatment effect. Additional efficacy analyses using NBR regression and different imputation schemes that were requested based on concerns regarding the initially employed methodology did not show a statistically significant treatment effect over placebo.The differences in SCC numbers between glutamine and placebo in those analyses ranged between -0.9 (favouring placebo) and 0.5, depending on the imputation scheme. Thus, the treatment effect varies greatly depending on the scheme, but is consistently small and not statistically significant across the imputation schemes. In the most realistic imputation scenario (scheme 3) the difference in the mean number of SCC was roughly 0.4.

An important uncertainty relates to SCC adjudication and related reliability/unbiasedness of study results: Data reveal a large discrepancy in the number of crises adjudicated by investigators vs. those adjudicated by a Central Adjudication Committee (CAC). The individual reasons for re-adjudicating crises have not been recorded. Importantly, even though the CAC reportedly acted unaware of treatment allocation, there is a noteworthy re-adjudication imbalance between treatment arms, favouring the glutamine arm: around twice as many SCC events (as determined by investigators) were overruled for the glutamine arm compared to the placebo arm (roughly 24% vs. 12%). The fact that 5 counts of major complications (i.e. priapism, splenic sequestration and ACS), for which relatively good diagnostic certainty has to be assumed, were overruled for the glutamine arm (vs. zero for placebo) is also critically noted in this context. This demands cautious interpretation and (primary) analyses based on investigator-adjudicated crisis counts are considered in addition to the CAC results to understand glutamine benefit. Respective point estimates show an even smaller effect.

Furthermore, information regarding treatment effect on incidence and severity of self-managed crises (which comprise a large proportion of crises experienced overall by SCD patients) is not available, which impedes a complete understanding of the effect pattern overall.

Some subgroup analyses (i.e., for age, dose, region) showed inconsistencies in efficacy results and these are difficult to interpret.

3.4. Unfavourable effects

Across the two main studies, four fatalities have been reported in the glutamine group and none for placebo (ISS); three of them died of cardiac arrest during sickle cell crises. Three serious thrombosis events occurred in the glutamine group and none in placebo. All patients experiencing serious thrombosis events took Hydroxyurea in combination with glutamine (and three of the four patients who died).

Rates of TEAEs were generally high in the clinical studies, which was attributed to the underlying disease. The most common drug related adverse events recorded in the clinical studies are gastrointestinal disorders (constipation (7.5%; placebo: 4.5%), nausea (2.7%; placebo: 0.9%), diarrhoea (1.6%; placebo: 0.9%), vomiting (1.6%; placebo: 2.7%)), abdominal pain upper (2.7%; placebo: 0.9%) and abdominal pain (2.1%; placebo: 3.6%), pruritus (1.1%; placebo: 0.9%), chest pain, hypersplenism, increased appetite, back pain, pain in extremity, headache and cough. These TEAEs were reported repeatedly as not severe TEAEs and were resolved without stopping the treatment with glutamine.

Patients aged ≤ 18 years of age reported some TEAEs more frequently compared with adults: Pain in extremities was reported in 22.5% of paediatric patients receiving glutamine compared to 8.3% for placebo; back pain was observed in 21.3% of children receiving glutamine and in 4.2% receiving placebo.

The number of patients who reportedly discontinued due to AEs is low, but higher in the glutamine group compared to placebo (5 vs. 1).

3.5. Uncertainties and limitations about unfavourable effects

The small number of cardiac events and related fatalities in the study sample render considerations on causality and potential relationship to treatment or other root causes difficult. A relation of the observed four deaths to study drug seems unlikely, but cannot be ruled out completely, which remains an important uncertainty. Notably, all three patients who died from cardiac arrest took concomitantly HU; one died four months after the end of the study.

No interaction studies have been performed with HU, but a considerable part of the study population (62.6%) used HU concomitantly with glutamine. Patients taking both were the only subgroup experiencing serious thrombosis events (all SAEs). Results further show three deaths for glutamine and HU compared to one with glutamine alone (and none for placebo), as well as higher rates in patients taking both for SCC, ACS and different pain events. Whether these observations are indeed caused by the concomitant medication use or whether this is a reflection of more severely affected patients being more likely to (i) receive more than one treatment for their disease and (ii) experience TEAEs cannot be fully elucidated with the available data.

The PK of glutamine and its metabolites (glutamate and ammonia) has not been studied in sickle cell patients and there is a risk that an altered metabolism may lead to pharmacologically relevant levels of glutamine metabolites (e.g. glutamate, ammonia), especially in patients with reduced biotransformation capacity.

Respective warnings for patients with renal and hepatic impairment were proposed in the product information.

"Pain in extremities" and "back pain" were reported (in an unbalanced fashion favouring placebo) by considerably more paediatric than adult patients.

In study GLUSCC09-01, the overall withdrawal rate is higher in the glutamine group compared to placebo. Consequently exposure and follow-up durations differ, which needs to be considered when comparing incidence rates between Xyndari and placebo arms.

Incidences of safety events of interest between more and less severely affected SCD patients or different genotypes cannot be assessed as these data are not available. Available data concerning the safety profile must be extrapolated to other disease groups/genotypes/ severities.

The safety database, though admittedly in an orphan condition, is relatively small and may not have allowed for the detection of infrequent, yet potentially important safety events or potentially vulnerable subgroups with sufficient granularity. This puts particular onus on external data, which is not systematically discussed and was poorly presented by the Applicant. Available literature is however mostly supportive of a benign glutamine safety profile. Presented literature indicates concerns for patients with hepatic and renal failure.

The necessity/usefulness of a tapering phase in case of treatment cessation is currently unknown as is the risk for infections after treatment discontinuation after long-term treatment with glutamine. There might be a need for dose adjustment of diabetes medication in patients receiving medication for the treatment of diabetes Type I and II, because literature indicates that glutamine could cause a reduction of blood glucose level

Finally, the safety of glutamine beyond one year in SCD patients remains uncertain.

3.6. Effects Table

Table 10: Effects Table for Xyndari in the treatment of sickle cell disease (SCD).

Effect	Short Description	Unit	Glutamine	Placebo	Strength of evidence	References
Favourable Effects						
Negative binomial regression (NBR) analysis (additionally requested analyses)						
Primary Endpoint – Sickle cell crisis (SCC)	NBR (controlling for strat.fact. region and HU use), *single imputation based on placebo completers*	mean per 48 weeks (95%CI)	3.54 (3.16, 3.96)	3.97 (3.44, 4.59)	Data from double-blind, RCT; Effect dependent on imputation method used; inconsistencies in effect across age cohorts, dose cohorts & regions; difference in effect between dataset based on CAC adjudication (presented here) vs. investigator	Pivotal study GLUSCC 09-01
		Rate Ratio (95%CI)	0.89 (0.75, 1.06) p = 0.193			

Effect	Short Description	Unit	Glutamine	Placebo	Strength of evidence	References
	NBR (controlling for strat. fact. Region, HU use), *multiple imputation based on placebo completers*		0.82 (0.65, 1.04) p = 0.092		adjudication; imputation was (unnecessarily) limited to placebo *completers* which may lead to an overestimation of effects;	
Secondary Endpoint - Hospitalisations	NBR, *multiple imputation based on placebo completers*		0.82 (0.62, 1.08) p = 0.156			
Secondary Endpoint - Emergency room visits	NBR, *multiple imputation based on placebo completers*		0.75 (0.51, 1.10) p = 0.142			
Additional Endpoint – Acute Chest Syndrome	NBR, *multiple imputation based on placebo completers*		0.41 (0.18, 0.92) p = 0.031			
Additional Endpoint - Cumulative duration of SCCs	NBR, *multiple imputation based on placebo completers*	mean days per 48 weeks (SD)	24.0 (25.84)	28.1 (33.16))		
		Rate Ratio (95%CI)	0.88 (0.63, 1.24) p = 0.464			
Primary Endpoint – Sickle cell crisis (SCC)	NBR (controlling for strat.fact. region and HU use), *single imputation based on placebo completers*	Rate per 48 weeks (95%CI)	4.15 (2.88, 5.99)	7.39 (5.17, 10.58)	Data from small, double-blind RCT; results vary throughout depending on imputation/stratification factors used for analysis; less than half of subjects completed study; difference in number of Hydroxyurea users between treatment arms (glutamine: 43%; placebo: 27%); one trial centre excluded from analysis by Applicant (based on presumed fraud), results change if included;	Phase II study 10478
		Rate Ratio (95% CI)	0.56 (0.36, 0.87) p = 0.012			
	NBR (controlling for strat. fact. Region, HU use), *multiple imputation based on placebo completers*		0.45 (0.22, 0.90) p = 0.025			

Effect	Short Description	Unit	Glutamine	Placebo	Strength of evidence	References
	NBR (controlling for strat. fact. Region, HU use, sex, age, baseline SCCs), *multiple imputation based on placebo completers*		0.36 (0.03, 4.52) p = 0.425			
Secondary Endpoint - Hospitalisations	NBR, *multiple imputation based on placebo completers*		0.67 (0.33, 1.35) p = 0.264			
Cochran–Mantel–Haenszel (CMH) test using ranks as scores (Pre-specified analyses)						
Primary Endpoint – Sickle cell crisis (SCC)	CMH, controlling for region and HU use, *single imputation based on mean values of completers of the respective treatment arm*	Mean per 48 weeks (SD)	3.2 (2.25)	3.9 (2.53)	See limitations listed above. p = 0.063	Pivotal study GLUSC C09-01
Primary Endpoint – Sickle cell crisis (SCC)	CMH, controlling for region and HU use, *single imputation based on mean values of completers of the respective treatment arm*	Mean per 48 weeks (SD)	4.5 (5.37)	10.8 (18.74)	See limitations listed above. p = 0.076	Phase II study 10478
Uncertainties: The CMH test does not provide estimates of the treatment differences that can easily be interpreted and the single imputation based on mean values of completers of the respective treatment arm is likely to have overestimated the treatment effect, due to a large and imbalanced number of patients discontinuing the study (more patients in the glutamine arm discontinued). No post-withdrawal SCC counts of these patients are available. The impact of missing data imputation is large.						
Unfavourable Effects[1)]						
Constipation	Drug related effect	% (N)	7.5%[2)] (14/187)	4.5% (5/111)	RCTs, Descriptive statistics only	ISS
Deaths	Other TEAE of interest	% (N)	2.1% (4/187)	0% (0/111)	RCTs, Descriptive statistics only	ISS

Effect	Short Description	Unit	Glutamine	Placebo	Strength of evidence	References
Thrombosis	Other TEAE of interest	% (N)	1.6% (3/187)	0% (0/111)	RCTs, Descriptive statistics only	ISS
Pain in extremities [3)]	Other TEAE of interest	% (N)	13.4% (25/187)	7.2% (8/111)	RCTs, Descriptive statistics only	ISS
Back pain[4)]	Other TEAE of interest	% (N)	12.3% (23/187)	5.4% (6/111)	RCTs, Descriptive statistics only	ISS
Chest pain	Other TEAE of interest	% (N)	12.3% (23/187)	8.1% (9/111)	RCTs, Descriptive statistics only	ISS
SAEs	Patients with at least 1 SAE	% (N)	75.4% (141/187)	80.2% (89/111)	RCTs, Descriptive statistics only	ISS
SAEs (drug related)	Patients with at least 1 **drug-related** SAE	% (N)	1.6% (3/187)	2.7% (3/111)	RCTs, Descriptive statistics only	ISS
Discontinuations due to TEAEs	Patients who permanently discontinued to **drug related** TEAE	% (N)	1.6% (3/187)	0 (0/111)	RCTs, Descriptive statistics only	ISS
Discontinuations due to SAEs	Patients who permanently discontinued to **drug related** SAE	% (N)	0.5% (1/187)	0 (0/111)	RCTs, Descriptive statistics only	ISS

Abbreviations:
TEAE: treatment- emergent adverse event
ISS: integrated safety analysis (studies 10478 and GLUSCC09-01)
N: number of patients

Notes:
[1)] TEAEs of special interest
[2)] As total TEAEs: treatment: 21.4%, placebo: 18.0%
[3)] Difference mainly seen in children: Children: 22.5% vs 8.3%; Adults: 6.5% vs 6.3%.
[4)] Difference mainly seen in children: Children: 21.3% vs 4.2%; Adults: 5.6% vs 6.3%.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

From a non-clinical and clinical perspective, significant uncertainty exists with respect to the proposed mode of action. The pharmacology of Xyndari has not been adequately characterised and the presented literature data does not enable a conclusive description of the PK profile. The absence of PK data in the intended target population is regarded an important shortcoming. Also a proper characterisation of dose-exposure-response relationship across the overall population and potentially meaningful subgroups is missing.

Sickle cell crises (SCCs) are considered a key manifestation of SCD. The primary endpoint of a reduced annual incidence of SCCs is acknowledged to be relevant for the patient. In the Xyndari studies, pain was the key driver for patients to seek help/visit the hospital and leading to SCC adjudication. However, the chosen

primary endpoint per definition only reflects "the tip of the iceberg". Many pain events severe enough to lead patients into hospital and being classified by treating physicians as SCC were not adjudicated as SCC (did not fulfil all criteria). These events still have a substantial impact on the QoL of SCD patients. Besides that, the majority of pain events in SCD are self-managed; however, no data on pain self-management is available. Notably, QoL was assessed in the phase II study and did not show any significant between-group differences.

With regards to the main outcomes from the single pivotal trial and the supportive study, efficacy has not been conclusively demonstrated. Pivotal study GLUSCC09-01 and supportive study 10478 both failed their pre-planned primary analyses. Had the primary analyses been successful, the interpretation of results would still have been complicated by an insufficient understanding of the impact that the observed differential dropout pattern, dealing with missing data, and the statistical methodology. Additionally requested analyses, and the missing data issue, also failed and furthermore confirm that the initially reported results likely overestimate glutamine efficacy. Overall, it cannot be excluded with sufficient certainty that Xyndari has no or a much smaller effect in terms of preventing SCCs than postulated by the Applicant. Post-hoc considerations/analyses applied on the same dataset by the Applicant ("integrated analysis") bear the risk of being data-driven and there are important reservations to relying on respective results for benefit/risk assessment.

Assuming a mean natural course of approximately four sickle cell crises per year (based on placebo data from SIKLOS and Xyndari studies; highly variable and highly dependent on the definition/adjudication applied as e.g. described by Smith & Scherer 2010), the observed reduction in SCC in quantitative terms is considered low, irrespective of which analysis and methodology are considered. The observed placebo rate, in addition to the fact that a large proportion of subjects was treated with prior and concomitant hydroxurea, may also suggest that a rather severely affected population has been studied. Other (large-scale) reports state a lower mean annual crisis frequency in SCD (Platt *et al.* 1991). The clinical relevance of such a small reduction in a less severe population with lower SCC frequency appears very questionable.

Secondary analyses suggest a reduced number of hospitalisations when treated with Xyndari, shorter cumulative duration of crises and a reduced incidence of acute chest syndrome (ACS), which are all deemed relevant to the patient.

In the presented studies, no data on pain self-management is available. Notably, QoL was assessed in the phase II study and did not show any significant differences between-group.

In summary, the benefit of Xyndari in terms of SCC prevention or other relevant clinical endpoint has not been demonstrated.

From a safety perspective, there are few concerns, even if a number of uncertainties remain. The majority of frequently observed safety signals, mainly related to the gastrointestinal system, appear manageable, but might necessitate stopping Xyndari treatment. Differential treatment discontinuation disfavouring the Xyndari arm became evident this being related to tolerability issues, at least for some subjects, cannot reliably be excluded based on the provided information. Consequently exposure and follow-up durations differ between study arms which need to be considered when comparing incidence rates between treatment and placebo arms.

Observed inconsistencies across subgroups (i.e., a higher incidence of pain in the paediatric Xyndari cohort compared to placebo, but no respective signal in adults) and the occurrence of four deaths in the Xyndari arms across the two main studies are noted. It is acknowledged that pain as well as cardiovascular events are frequent manifestations of SCD, and mortality is increased in SCD patients compared to the general population. Thus, these findings may be interpreted as disease related rather than as an actual safety signal.

Nonetheless, the mortality signal remains an uncertainty. Similar considerations apply for the recorded difference in thrombosis events between study arms (in patients concomitantly taking HU).

The metabolism of glutamine involves both the liver and kidneys; there may be a (potentially dose-dependent) risk for the formation of pharmacologically relevant levels of glutamine metabolites (e.g. glutamate, ammonia) in patients with organ impairment.

3.7.2. Balance of benefits and risks

With regards to the main outcomes from the single pivotal trial and the supportive study, efficacy has not been conclusively demonstrated.

The known and observed safety profile of glutamine raises few and mostly reversible or manageable concerns in general. The observed imbalance in deaths and thrombosis events remains an uncertainty even if relatedness of these events with Xyndari is considered unlikely. Incidences of pain episodes were notably increased with glutamine in younger patients, which is not fully understood.

In summary, based on the totality of non-clinical and clinical evidence submitted, it is concluded that efficacy has not been established. Therefore, the balance of benefits and risks cannot be considered positive.

3.8. Conclusions

The overall B/R of Xyndari is negative.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Xyndari in the prevention of sickle cell crises in adults and children 5 years and older suffering from sickle cell disease, the CHMP considers by consensus that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product.

The CHMP considers that:

- The single pivotal phase 3 study failed the primary analysis pre-specified in the statistical analysis plan. Additionally, there was a high level of missing data due to drop-out with a higher number in the glutamine treatment. It cannot be assumed that reasons for study discontinuation were non-informative or unrelated to lack of efficacy or safety. Data on SCC was not collected after drop-out. Thus, the impact of missing data and imputation strategies was carefully considered using sensitivity analyses. Applying plausible assumptions and suitable imputation methods, efficacy could not be convincingly demonstrated. This was even more evident from the relevant analyses based on investigator's adjudication of SCC events.

- The observed effects were not consistent between different subgroups (e.g. across age groups and weight/dose strata) and there was no clear corroboration from analyses of other endpoints. The phase 2 study also had important limitations due to the small size of the study, the high variability, the high drop-out rate and the confounding due to an imbalance in hydroxyurea use between treatment arms. Thus, there is no convincing supportive data to corroborate the claimed effects on SCC and number of hospitalisations.

- In summary, based on the totality of the non-clinical and clinical evidence submitted, it is concluded that the efficacy has not been established. Therefore, it is not possible to establish a positive benefits risk balance for glutamine.

the CHMP is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, the efficacy of the above mentioned medicinal product is not properly or sufficiently demonstrated.

Therefore, the CHMP has recommended the refusal of the granting of the marketing authorisation for Xyndari.