

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

208587Orig1s000

NON-CLINICAL REVIEW(S)

Memo-to-File

Date: June 6, 2017

From: Shwu-Luan Lee, Ph.D.

NDA: 208587, SDN 0020 (eCTD 0019; also cross reference to Day 74 letter)

Sponsor: Emmaus Medical Inc.

Subject: Evaluation of Carcinogenicity Assessment Document (CAD) for oral L-Glutamine

Regulatory Recommendation: To accept the Applicant's request for waiver of requirement for carcinogenicity assessment in animals

Introduction

Emmaus Medial, Inc. submitted NDA 208587 for L-glutamine for the indication of treatment for sickle cell disease (SCD). This is a 505(b)(2) NDA with the Applicant's own NutreStore (NDA 21667, for short bowel syndrome) as the listed drug. No carcinogenicity assessment of L-glutamine was required for the approval of NDA 21667, due to the short treatment period. In patients with SCD however, treatment with L-glutamine will be longer (i.e., chronic), thus evaluation of potential of L-glutamine to be carcinogenetic is warranted. The Applicant has submitted a CAD in lieu of 2-year bioassay data in rodents, to support their request for a waiver of the requirement to conduct carcinogenicity evaluations in animals.

Summary

The Pharmacology/Toxicology review team recommends accepting the Applicant's request for the waiver to conduct animal carcinogenicity assessment for oral L-glutamine. The recommendation is based on the following rationale:

- Human experience: L-glutamine is well characterized chemically, and considered to be a conditionally essential amino acid. There has been over seven decades of experience with L-glutamine as a dietary supplement. Of the clinical studies described in the published literature, a range of L-glutamine doses were used, including doses similar to the proposed oral L-glutamine dose of 30 g/day. L-glutamic acid, formed from L-glutamine in human bodies, and its various salt forms are used as nutrients added to foods for human consumption.
- Mechanism of action: L-glutamine serves as an anti-oxidant in sickle red blood cells (RBCs) to elevate the NAD redox potential, thereby countering the oxidant-dependent pathophysiology of sickle RBCs, such as sickle RBC adhesion to endothelium and the resulting vascular occlusion.
- No evidence indicating immunosuppressive, hormone perturbing potential of L-glutamine and L-glutamate (the main metabolite in the body), nor histopathological findings of hyperplasia in chronic studies in animals.

- L-glutamine and L-glutamate (the two compounds are interconverted metabolically in vivo) are negative in Ames test and/or chromosome aberration assay in mammalian cells.^{1, 2}
- No safe and effective therapy is available yet for the treatment of SCD. Patients with SCD are usually treated with chemotherapeutic agents such as hydroxyuria.

Background

L-glutamine has been approved under the trade name NutreStore® since 2004 for the treatment of short bowel syndrome (in combination with recombinant human growth hormone) at a dose of 30 g/day for up to 16 weeks.

Drug intended target/pharmacology

L-glutamine is an essential amino acid found in abundance in the human body. L-glutamine's biological roles include: 1) a precursor of nucleic acids and nucleotides such as the pyridine nucleotides, NAD and its reduced form NADH, 2) a preferred fuel for rapidly dividing cells including hematopoietic cells, and 3) a precursor for glutathione (GSH).

Genetic toxicology

L-glutamine showed no mutagenic activity in the bacterial reverse mutation assay (313-5000 µg/plate), and did not induce chromosomal aberrations in Chinese hamster lung fibroblast cells in the in vitro chromosomal aberration assay (Wong et al., Food Chem Toxicol 49: 2096-2102, 2011). Related glutamates, such as monosodium glutamate (MSG), were demonstrated negative in Ames tests (Zeiger et al., Environmental & Molecular Mutagenesis, 19: Suppl 21: 2-1412, 1992).

L-glutamine was non-genotoxic based on the results of in vitro and in vivo genotoxicity studies (the Joint FAO/WHO Expert Committee on Food Additives/JECFA, 2006).

Results of non-rodent chronic study

In addition to the toxicology studies reviewed under NDA 21667 for listed drug (LD) NutreStore (see attached review of NDA 208587 for highlights of the results), the Applicant cited results of two GLP 13-week repeated dose toxicity studies in rats. According to Tsubuku et al., 2004³ L-glutamine in feed at doses up to 5% of the diet for 13 weeks was tolerated (approximating doses of 33-39 g/day for a 60 kg human). Slightly decreased body weight gains and increase urinary protein were observed at L-glutamine levels of ≥ 2.5 % diet. In another study by Wong et al., 2011⁴ the NOAEL was 5% diet (approximating 3832-4515 mg/kg/day for male and female rats, or 38-45 g for a 60 kg human). No remarkable histopathological findings were observed in either studies.

¹ Wong et al., Food Chem Toxicol 49: 2096-2102, 2011

² Ziegler et al., J Parenteral Enteral Nutr. 14: 137S-146S, 1990

³ Tsubuku et al., Int J Toxicol 23: 107-112, 2004

⁴ See footnote 1

Exposure margins in chronic toxicology studies

The maximum recommended human dose of L-glutamine in NDA 208587 is 30 g/day, which is in the range of the tolerated doses reported in two 13-week repeated dose toxicology studies in rats.

Metabolic profile (humans)

(The following is adapted based on Wong et al, 2011)

Supplemental glutamine does not undergo significant hydrolysis in the stomach or upper duodenum and is available for absorption within the intestine (Wu et al., 1996).⁵ After absorption, L-glutamine is extensively metabolized to citrulline, arginine, glutamine, and proline.⁶ Entry of glutamine in the portal circulation is approximately one-third of the ingested dose and is largely taken up in the liver for the synthesis of glutathione, urea, and acute phase proteins, as well as gluconeogenesis.⁷ Any remaining glutamine that enters the systemic circulation is rapidly metabolized.⁸ Given the extensive utilization of glutamine by the small intestine and liver, only minimal amounts of ingested glutamine would remain intact and enter the systemic circulation. The metabolic fate of glutamine suggests no safety concern associated with dietary supplementation of this amino acid.

Evidence of hormone perturbation

Unlikely, based on mechanism of action and toxicology data.

Immune suppression

Unlikely, based on mechanism of action and toxicology data.

Special studies:

2-year carcinogenicity studies in mice and rats with L-glutamic acid and MSG (L-monosodium 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).

Transgenic mouse study, knock-out or animal disease models

Not applicable.

Applicant's question (response to Day 74 letter, request for CAD)

Does the Agency concur that based on the weight of evidence presented herein a waiver for conducting rodent carcinogenicity studies with L-glutamine is acceptable?

FDA response:

Yes, we concur.

⁵ Wu et al., J Nutr. 126: 2578-2584, 1996

⁶ Reeds and Burrin, J Nutr. 131: 25055-25085, 2001

⁷ Coster et al., Asia Pac. J Clin Nutr 13: 25-31, 2004

⁸ Ziegler et al., JPEN J Parenter Enteral Nutr. 14 (suppl): 1375-1465, 1990

Update of PTCC-eCAC response

The Pharmacology Toxicology Coordinating Committee (PTCC) Executive Carcinogenicity Assessment Committee (eCAC) agrees with the Division's decision of no additional carcinogenicity studies.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SHWU LUAN LEE
06/06/2017

CHRISTOPHER M SHETH
06/06/2017

Memo-to-File

Date: June 4, 2017

From: Shwu-Luan Lee, PhD

NDA: 208587 (SDN 001)

Product: L-glutamine

Sponsor: Emmaus Medical Inc.

Subject: Review of additional toxicology data which is included in the Carcinogenicity Assessment Document (CAD) submitted on March 24, 2017

Introduction

Several general toxicity and genotoxicity studies from the scientific literature were submitted with the Carcinogenicity Assessment Document for L-glutamine (GLN; Gln), to support the Applicant's request for waiver of the requirement to conduct bioassays in animals to assess for the carcinogenicity of L-glutamine, additional toxicology studies of general toxicity and genotoxicity are included. This data and additional data submitted on monosodium glutamate (MSG) and its related compounds are reviewed and summarized in this memorandum.

General toxicology

Article title: Thirteen-week oral toxicity study of L-glutamine in rats (Tsubuku et al., Int J Toxicol. 23: 107-112, 2004) (GLP)

Key findings:

- L-glutamine administered to rats in feed at up to 5% of the diet for 13 weeks was tolerated (approximating L-glutamine doses of 33-39 g/day for a 60 kg human).
- Slightly decreased body weight gains and increased urinary protein were observed at L-glutamine doses ≥ 2.5 % diet.
- The NOEL is 1.25% diet, which is equivalent to 833 mg/kg/day or 4998 mg/m²/day in males and 964 mg/kg/day or 5784 mg/m²/day in females (approximating 8-9 g/day in a 60 kg human).

The toxicological effects of L-glutamine were evaluated in male and female Sprague-Dawley rats. produced by [REDACTED]^{(b) (4)} was incorporated into a standard diet at 1.25%, 2.5%, and 5.0% (w/w), respectively (n=12/sex/group). A control group of rats received only a standard diet (n=6/sex/group). The administration period was followed by a 5-week recovery period (control and high dose groups), during which only the standard diet was provided to all animals.

Results:

The average L-glutamine intake for males and females in each group is outlined in the table below. Of note, the highest level of L-glutamine in feed approximates the maximum recommended human dose of 30 g/day for a 60 kg subject.

Table 1: Percent L-glutamine in diet and estimated doses in rats and humans (HED) (Tsubuku et al., 2004)

Table from the Applicant; CAD

	Average GLN dose (mg/kg/d)		GLN dose (mg/m ² /d)		HED (mg/kg/d) ^a		HED (g/d) 60 kg subject ^a	
	Male	Female	Male	Female	Male	Female	Male	Female
Group I Control Diet	0	0	0	0	0	0	0	0
Group II 1.25% GLN-Diet	833	964	4,998	5,784	134	156	8	9
Group III 2.5% GLN-Diet	1,654	1,984	9,924	11,904	267	320	16	19
Group IV 5.0% GLN-Diet	3,379	4,026	20,274	24,156	545	649	33	39

All rats survived through the treatment and recovery period. There were no remarkable treatment-related findings in clinical signs, hematology, clinical chemistry, or gross and histopathology.

- Body weight: Slightly reduced body weight gains were observed in Group IV; however, the finding resolved.

Table 2: Changes in body weight gains (Tsubuku et al., 2004)

	Males		Females	
	1-13 weeks	1-5 weeks (R)	1-13 weeks	1-5 weeks (R)
Control (standard diet)	376±49 g	44±11 g	162±19 g	21±15 g
Group IV (Gln 5%)	360±47 g	46±19 g	152±18 g	16±11 g

R: recovery period

- Urinalysis: All findings resolved.

Table 3: Urinalysis findings at end of 13 weeks (Tsubuku et al., 2004)

	Males			Females		
	Control	2.5%	5%	Control	2.5%	5%
Urine protein*	0	5	5	1	0	4

*Incidence (out of n=12/group)

(The following statement is excerpted from the article; other than urine protein, there are no additional data included in the article.)

A tendency toward an increase in the number of positive incidences for urine protein was observed in females in the 2.5% and 5.0% groups; however, total protein in the blood did not increase, and no pathological changes in the tissue of the kidneys or the urinary tracts were observed. L-glutamine triggered slight decline in a urine pH, suggesting a slight acidifying effect within the urinary tract. An increase in the number of positive incidence of ketone bodies was also observed in the 2.5% and 5.0% groups. Nevertheless, ketosis was not found.

Article title: Oral subchronic and genotoxicity studies conducted with the amino acid, L-glutamine (Wong et al., Food and Chemical Toxicology, 49: 2096-2102, 2011) (GLP-compliant Japanese Good Laboratory Practice Regulations of Animal Studies on Feed Additives)

Key findings:

L-glutamine in feed levels up to 5% diet (estimated 3832-4515 mg/kg/day for male and female rats, or 38-45 g in 60 kg human subject) for 13 weeks was tolerated. The dose level of 3832-4515 mg/kg/day is also the NOAEL of the study.

The toxicological effects of L-glutamine were evaluated in male and female Sprague-Dawley rats. L-glutamine produced by (b) (4) was incorporated into a standard diet at 0.5%, 2.5%, and 5.0% (w/w), respectively (n=10/sex/group).

Results

Table 4: Dietary intake of L-glutamine in rats during 13-week toxicity study (Wong et al., 2011)

Table from the Article

Test group ^a	Dietary treatment	Mean daily intake of L-glutamine over 13-week study period (mg/kg bw/day) ^b	
		Males	Females
Control	Lab MR Stock ^c	0	0
Low-dose	Lab MR Stock + 0.5% L-glutamine	379	439
Mid-dose	Lab MR Stock + 2.5% L-glutamine	1867	2234
High-dose	Lab MR Stock + 5% L-glutamine	3832	4515

^a Ten animals per test group.

^b Values for intake of supplemental L-glutamine only.

^c Average L-glutamine content of 0.0071%.

All rats survived through the 13-week study period. No remarkable findings were noted for any in-life observations or at gross or histopathological examination.

Genotoxicity¹

The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) concluded that L-glutamine was non-genotoxic based on the results of in vitro and in vivo genotoxicity studies.

Article title: Oral subchronic and genotoxicity studies conducted with the amino acid, L-glutamine (Wong et al., Food and Chemical Toxicology, 49: 2096-2102, 2011) (GLP)

Key findings:

- L-glutamine was not mutagenic in the Ames test.
- L-glutamine was not clastogenic in CHL/IU cells.

Ames test:

Method	pre-incubation procedure
Test stains	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, and TA1537 <i>E. coli</i> mutant Wp2 <i>uvrA</i>
Control	Vehicle (negative) control: 0.5% carboxymethylcellulose (CMC) solution Positive controls: With S-9: TA98, TA100, TA1535 and TA1537 and WP2 <i>uvrA</i> : 2-aminoanthracene (2-AA) Without S-9: TA98, TA100, and WP2 <i>uvrA</i> : 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), TA1535: sodium azide, TA 1537: 9-aminoacridine
Concentrations	313, 625, 1250, 2500, and 5000 µg/plate in the presence and absence of metabolic activation [S9 microsomal fraction (S9 mix)]
Definition of positive results	A two-fold or greater dose-dependent increase or a reproducible increase in the mean number of revertants compared to the negative control.
Validity of the study	<ul style="list-style-type: none">○ Positive controls induced a greater than two-fold increase in the number of revertant colonies,○ Revertants in the negative controls were similar to historical negative control data.

Result:

L-glutamine did not increase the mean number of revertant colonies in any test strain at any concentration tested (313-5000 mg/plate), either in the presence or absence of metabolic activation.

Chromosomal aberration test

Method	See table below
Cells	Chinese hamster lung (CHL/IU) cell line

¹ Several studies in the literature have reported that L-glutamine is able to prevent genetic damage in mice and rats exposed to genotoxic agents both *in vivo* and *in vitro*. These studies are not discussed in the memo-to-file.

Control	Vehicle (negative) control: 1% carboxymethylcellulose (CMC) solution Positive controls: With S-9: 3,4-benzo[a]pyrene (BP). 10 µg/mL Without S-9: 1-methyl-3-nitro-1-nitrosoguanidine (MMC, 2.5 µg/mL)
Concentrations	153, 313, 625, 1250, 2500, and 5000 µg/mL, see below
Definition of positive results	There was a significant or dose-dependent increase or a reproducible increase in the frequency of chromosomal aberrations compared to the solvent control. A P-value of < 0.05 was considered to be statistically significant.
Validity of the study	The positive control agents produced a significant increase in the percentage of cells with chromosome damage.

Table 5: The condition and concentrations (Wong et al., 2011)

Treatment condition	Treatment time	Recovery time	Concentrations (µg/mL)
Without S9	6 hr	18 hr	625, 1250, 2500, and 5000
	24 hr or 48 hr	0 hr	313, 625, 1250, 2500, and 5000
With S9	6 hr	18 hr	625, 1250, 2500, and 5000

Cytotoxicity was observed following continuous treatment with L-glutamine at a concentration of 5000 µg/mL for 24 and 48 h in which cell growth was reduced to 64.5 and 50.5% of the negative control, respectively.

To assess for the induction of structural chromosomal aberrations, 200 metaphase cells (100 metaphases/plate) from each group were examined using a microscope. Structural chromosomal aberrations including chromatid-type aberrations (breaks and exchanges), chromosome-type aberrations (gaps, breaks, dicentrics, and rings), and numerical aberrations (polyploidy) were counted.

Result:

In the chromosomal aberration experiments, there were no significant differences in the incidence of CHL/IU cells displaying chromosomal aberrations following short-term or continuous treatment with L-glutamine at any concentrations tested at any time point assessed, either in the presence or absence of metabolic activation.

Article title: Protective effects of the amino acid glutamine and of ascorbic acid against chromosomal damage induced by doxorubicin in mammalian cells (Tavares et al., *Teratogenesis, Carcinogenesis, and Mutagenesis* 18:153–161, 1998)

Key findings

In studies conducted in vitro (CHO cells) or in vivo (rats, bone marrow cells), the treatments with L-glutamine and ascorbic acid (AA) did not induce chromosomal

aberrations, while increased frequencies of chromosomal aberrations were observed in the DXR-treated cultures.

Results

Table 6: Chromosomal aberrations and mean mitotic index in CHO cells (Tavares et al., 1998)

(Table from the article)

Treatment (µg/ml)	Chromosomal aberrations										Total/ 100 cells	AM (%)	
	MI (%)	Gaps C	Breaks C	IC	E	T	Q	D	R	Total			
Control	10.18	7	8	1	0	0	0	0	0	0	16	2.7	5.3
GLN (292.2)	8.82	5	4	0	0	0	0	0	0	0	9	1.5	3.0
AA (50)	10.15	9	3	0	0	0	0	0	0	0	12	1.0	4.0
DXR (1.2)	1.10*	108	208	8	31	5	5	9	2		376*	62.7*	59.7
DXR+ GLN	1.40*	86	156	21	32	4	6	10	4		319***	53.2***	60.3
DXR + AA	1.45*	95	170	7	25	4	3	4	4		312***	52.0***	56.0
DXR + GLN + AA	1.03*	105	170	11	27	5	1	5	3		328***	54.7***	58.3

* $P < 0.05$ compared with negative control.

** $P < 0.05$ compared with the DXR treatment.

AA = ascorbic acid; AM = abnormal metaphases; C = chromatid; CHO = Chinese Hamster Ovary; D = dicentric; DXR = doxorubicin; E = complex exchange; GLN = L-glutamine; IC = isochromatid; MI = mitotic index; Q = quadriradial figure; R = ring; T = triradial figure.

^a One hundred metaphases were analyzed per culture, for a total of 300 cells per treatment.

Source: Tavares et al. 1998

The chromosomal aberrations detected at highest frequency were chromatid breaks, followed by isochromatid breaks and other complex rearrangements such as complex exchanges and triradial and quadriradial figures.

Similar results were observed in the study with rat bone marrow cells (see below, in vivo studies).

Genotoxicity evaluation of L-glutamate and other glutamates

Monosodium glutamate (10 to 20000 µg/plate) was negative when tested in *Salmonella typhimurium* strains TA98, TA100, and TA1538 with and without metabolic activation (Chemical Carcinogenesis Research Information System Database 2017). In a subsequent study, MSG (100 to 10000 µg/plate) dissolved in water was not mutagenic to *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 with or without metabolic activation.²

In vivo studies

² Zeiger et al., Environmental & Molecular Mutagenesis 19: Suppl21:2-141, 1992

There are no in vivo studies for L-glutamine alone. The effect of L-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; see above).

Table 7: Chromosomal aberrations and mean mitotic index in rat bone marrow cells (Tavares et al., 1998)

(Table from the article)

Treatment (mg/kg b.w.)	MI (%)	Chromosomal aberrations									Total	Total/100 cells	AM %
		Gaps C	Breaks										
		C	C	IC	E	T	Q	D	R				
Control	3.4	1	12	3	0	0	0	0	0	16	2.7	2.7	
GLN (600)	2.2	1	9	10	0	0	0	0	0	20	3.3	3.2	
AA (200)	3.3	0	6	0	0	0	0	0	0	6	1.0	1.0	
DXR (90)	1.7*	31	236	48	14	5	6	0	0	340*	56.7*	37.7	
DXR + GLN	2.5	17	142	25	10	5	2	2	0	203***	33.8***	25.3	
DXR + AA	2.0*	22	149	33	8	6	2	0	1	220***	36.7***	27.0	
DXR + GLN + AA	2.1	24	146	48	14	13	6	1	0	252***	42.0***	29.3	

* $P < 0.05$ compared with negative control.

** $P < 0.05$ compared with the DXR treatment.

One hundred metaphases were analyzed per animal, for a total of 600 cells per treatment.

AA = ascorbic acid; AM = abnormal metaphases; b.w. = body weight; C = chromatid; D = dicentric; DXR = doxorubicin; E = complex exchange; GLN = L-glutamine; IC = isochromatid; MI = mitotic index; Q = quadriradial figure; R = ring; T = triradial figure.

Source: Tavares et al. 1998

L-glutamine and ascorbic acid (AA) administered separately, or in combination, significantly reduced the frequency of chromosomal aberrations induced by doxorubicin (DXR) ($P < 0.05$) by 40%, 35%, and 26%, respectively. DXR treated animals and those treated with ascorbic acid and DXR showed a significantly lower MI when compared to the negative control ($P < 0.05$).

Article title: Pretreatment with glutamine reduces genetic damage due to cancer treatment with cisplatin (Oliveira et al., Genet Mol Res., 12(4):6040-6051, 2013)

Key findings:

- Negative results were observed in peripheral blood samples collected from mice receiving oral doses of L-glutamine (150, 300 and 600 mg/kg) in the micronucleus assay and comet assay.
- Pretreatment of oral L-glutamine significantly alleviated the clastogenic (micronucleus assay) and genotoxic (Comet assay) damages of cisplatin.

The effects of enteral supplementation of glutamine in clastogenic damages caused by the acute administration of cisplatin were evaluated. Male Swiss mice were divided into eight groups ($n = 5$):

Table 8: Experimental design (Oliveira et al., 2013)

Table from the Applicant, CAD

Group	Treatment
G1 (control)	2 doses PBS 0.1 mL/10 g b.w.
G2 (cisplatin)	1 dose PBS 0.1 mL/10 g b.w. and 1 dose cisplatin 6 mg/kg b.w., ip
G3 (L-glutamine)	1 dose PBS 0.1 mL/10 g b.w. and 1 dose L-glutamine 150 mg/kg b.w., oral
G4 (L-glutamine)	1 dose PBS 0.1 mL/10 g b.w. and 1 dose L-glutamine 300 mg/kg b.w., oral
G5 (L-glutamine)	1 dose PBS 0.1 mL/10 g b.w. and 1 dose L-glutamine 600 mg/kg b.w., oral
G6 (Pre-treatment)	L-glutamine 150 mg/kg b.w., oral 24 hours before cisplatin 6 mg/kg b.w., ip
G7 (Pre-treatment)	L-glutamine 300 mg/kg b.w., oral 24 hours before cisplatin 6 mg/kg b.w., ip
G8 (Pre-treatment)	L-glutamine 600 mg/kg b.w., oral 24 hours before cisplatin 6 mg/kg b.w., ip

b.w. = body weight; ip = intraperitoneally; PBS = phosphate-buffered saline.

Source: Oliveira et al. 2013

Blood samples from each experimental group were collected for evaluation of mutagenicity and anti-mutagenicity (micronucleus assay) by tail vein puncture at 3 different time points (T0 = prior to drug or vehicle administration; T1 = 24 h after first L-glutamine or vehicle administration; T2 = 48 h after first L-glutamine or vehicle administration). At T2, samples were also collected for evaluation of DNA damage (comet assay)

Results:Micronucleus assay

At T2 (48 hours), L-glutamine at the oral dose of 300 mg/kg induced statistically significant increases of frequency of micronucleus formation when compared with the vehicle control. The toxicological significance of these findings is unclear given the lack of dose-dependence.

Cisplatin induced significant increases in the frequencies of micronucleus formation compared with the vehicle control at T1 (24 hours after treatment) and T2 (48 hours after treatment). Pretreatment of L-glutamine significantly reduced the clastogenic effect of cisplatin (G6, G7, and G8). The damage reduction percentage (DR %) was 90.0%, 47.3%, and 37.3% at T1 and 46.0%, 38.6%, and 34.7% for the doses of 150, 300, and 600 mg/kg body weight, respectively. The protective effect of L-glutamine was not dose dependent.

Table 9: Frequency of micronuclei in response to L-glutamine and cisplatin (Oliveira et al., 2013)

(Table from the article)

Treatment	Frequency of MN			Means \pm SD			DR%	
	T0	T1	T2	T0	T1	T2	T1	T2
G1	43	23	22	8.60 \pm 3.05	4.60 \pm 1.82	4.40 \pm 4.00	-	-
G2	29 ^a	133 ^{a*}	198 ^{a*}	5.80 \pm 2.39	26.60 \pm 4.04	39.60 \pm 4.98	-	-
Mutagenicity								
G3	20 ^{a*}	23 ^a	19 ^a	4.00 \pm 2.55	4.60 \pm 2.30	3.80 \pm 0.84	-	-
G4	20 ^{a*}	20 ^a	39 ^{a*}	4.00 \pm 0.71	4.00 \pm 2.24	7.80 \pm 3.70	-	-
G5	19 ^{a*}	16 ^a	22 ^a	3.80 \pm 0.84	3.20 \pm 2.28	4.40 \pm 1.14	-	-
Antimutagenicity								
G6	9 ^{b*}	34 ^{b*}	117 ^{b*}	1.80 \pm 0.84	6.80 \pm 3.35	23.40 \pm 8.08	90.0	46.0
G7	17 ^{b*}	81 ^{b*}	130 ^{b*}	3.40 \pm 1.14	16.2 \pm 8.04	26.00 \pm 5.29	47.3	38.6
G8	14 ^{b*}	92 ^{b*}	137 ^{b*}	2.80 \pm 1.64	18.4 \pm 2.70	27.40 \pm 10.55	37.3	34.7

* Statistically significant difference (Chi-squared test; $P < 0.05$).

b.w. = body weight; DR% = damage reduction percentage; G1 = control; G2 = cisplatin (6.0 mg/kg); G3 = L-glutamine (150 mg/kg b.w.); G4 = L-glutamine (300 mg/kg b.w.); G5 = L-glutamine (600 mg/kg b.w.); G6 = cisplatin (6.0 mg/kg) + L-glutamine (150 mg/kg b.w.); G7 = cisplatin (6.0 mg/kg) + L-glutamine (300 mg/kg b.w.); G8 = cisplatin (6.0 mg/kg)+L-glutamine (600 mg/kg b.w.); MN = micronucleus; SD = standard deviation; T0 = prior to drug or vehicle administration; T1 = 24 h after first L-glutamine or vehicle administration; T2 = 48 h after first L-glutamine or vehicle administration.

^a Compared statistically to the G1

^b Compared statistically to the G2

Comet assay

The results from the comet assay showed that L-glutamine did not have a genotoxic effect. Treatment with L-glutamine significantly reduced the frequency of basal damage compared to the control group. In terms of anti-genotoxicity, L-glutamine pretreatment was effective in reducing damage caused by chemotherapy treatment. The DR% was 113.0%, 117.4%, and 115.0% for the doses of 150, 300, or 600 mg/kg L-glutamine body weight, respectively.

Table 10: Frequency of damaged cells (DNA migration in the Comet assay) (Oliveira et al., 2013)

(Table from the article)

Treatment	Means ± SD							DR %
	Total ^a	Frequency ^b	Classes of DNA Damage				Score	
			0	1	2	3		
G1	81	16.20 ± 8.04	83.80 ± 8.04	16.20 ± 8.04	0.00 ± 0.00	0.00 ± 0.00	16.20 ± 8.04	-
G2	380	76.00 ± 7.00 ^{c*}	24.00 ± 7.00	74.8 ± 6.18	1.20 ± 1.30	0.00 ± 0.00	77.20 ± 7.95	-
Genotoxicity								
G3	22	4.40 ± 20.07 ^{c*}	95.60 ± 2.07	4.00 ± 2.07	0.00 ± 0.00	0.00 ± 0.00	4.40 ± 2.07	-
G4	15	3.00 ± 1.87 ^{c*}	97.00 ± 1.87	3.00 ± 1.87	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 1.87	-
G5	25	5.00 ± 3.35 ^{c*}	95.00 ± 3.89	5.00 ± 3.39	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 3.39	-
Antigenotoxicity								
G6	41	8.20 ± 3.27 ^{d*}	91.8 ± 3.27	8.20 ± 3.27	0.00 ± 0.00	0.00 ± 0.00	8.20 ± 3.27	113.0
G7	29	5.80 ± 1.30 ^{d*}	94.20 ± 1.30	5.80 ± 1.30	0.00 ± 0.00	0.00 ± 0.00	5.80 ± 1.30	117.4
G8	36	7.20 ± 1.79 ^{d*}	92.80 ± 1.79	7.20 ± 1.79	0.00 ± 0.00	0.00 ± 0.00	7.20 ± 1.79	115.0

* Statistically significant difference (Student t-test; $P < 0.05$).

b.w. = body weight; DNA = deoxyribonucleic acid; DR% = damage reduction percentages; G1 = control; G2 = cisplatin (6 mg/kg); G3 = L-glutamine (150 mg/kg b.w.); G4 = L-glutamine (300 mg/kg b.w.); G5 = L-glutamine (600 mg/kg b.w.); G6 = cisplatin (6.0 mg/kg) + L-glutamine (150 mg/kg b.w.); G7 = cisplatin (6.0 mg/kg) + L-glutamine (300 mg/kg b.w.); G8 = cisplatin + L-glutamine (600 mg/kg b.w.); SD = standard deviation.

^a Total number of damaged cells for each treatment.

^b Mean number of damaged cells by treatment.

^c Compared statistically to the G1.

^d Compared statistically to the G2.

Carcinogenicity (L-glutamine and glutamates)

Several lines of evidence indicate the carcinogenic potential of L-glutamine is likely low:

- L-glutamine supplementation does not stimulate tumor growth (Bartlett et al. 1995; Buchman 2001)
- L-glutamine can inhibit tumor cell proliferation (Yoshida et al. 1995; Liu et al. 2000), or decrease tumor size (Fahr et al. 1994; Klimberg et al. 1996; Shewchuk et al. 1997; Liu et al. 2000)
- L-glutamine increases natural killer cell (NK) activity against malignant cells (Fahr et al. 1994; Klimberg et al. 1996).

Shewchuk and colleagues studied the effect of dietary L-glutamine supplementation in tumor bearing female Sprague-Dawley Buffalo rats.³ The study results indicated that a purified diet supplemented with 20 g/kg L-glutamine reduced the growth of the Morris Hepatoma 7777 in both sedentary and exercise-trained rats.

In tumor bearing rats, L-glutamine supplement is beneficial in preventing deficiencies of L-glutamine and glutathione and in improving protein metabolism in the rats, without promoting growth of tumors.⁴ In a separate study in tumor bearing Fischer rats, Inoue et

³ Shewchuk et al., J Nutr. 127(1):158-166, 1997

⁴ Yoshida et al., JPEN J Parenter Enteral Nutr 19(6): 492-497, 1995

al. concluded that provision of L-glutamine-enriched diets to the tumor-bearing rats may maintain hepatic L-glutamine levels and prevent host L-glutamine depletion.

Kaufmann and colleagues demonstrated that dietary L-glutamine reduced DMBA (7,12-dimethylbenz[a]anthracene)-induced mammary tumorigenesis by 50%. This reduction was related to increased circulating and target tissue GSH concentrations. Importantly, it correlated most with differences in gut GSH fractional release, which DMBA disturbed while L-glutamine normalized.⁵ This group of researchers reported in a separate publication that oral L-glutamine supplementation enhances blood GSH content, gut GSH release, and NK activity, and prevents DMBA-induced tumor development.⁶

L-Glutamate and related glutamates

Although the carcinogenicity of L-glutamine itself has not yet been evaluated via bioassays in rodents according to the ICH S1 guideline, there are available 2-year carcinogenicity bioassay data in mice and in rats of L-glutamate, that is metabolically interconvertible in vivo with L-glutamine, and its related compounds, such as L-glutamic acid, and MSG (L-monosodium glutamate, and DL-monosodium glutamate).⁷

Carcinogenicity of MSG

Article title: Lack of carcinogenicity of monosodium L-glutamate in Fischer 344 rats (Shibata et al., Food Chem Toxicol. 33(5):383-391, 1995)

Key findings

- MSG was not carcinogenic in Fischer 344 rats following dietary administration of up to 5% MSG (corresponding to 1982 mg/kg/day in males and 2311 mg/kg/day in females) for 2 years.
- Non-neoplastic findings included non-dose dependent increased incidences of transitional cell hyperplasia of the renal pelvis.

Monosodium glutamate (MSG, 0, 0.6, 1.25, 2.5, and 5.0% in the diet) was administered to Fischer 344 rats (N = 50/sex/dose) for 2 years to assess its carcinogenicity potential. Corresponding mg/kg/day doses during Weeks 1-104 are as follows:

	Males				Females			
% diet	0.6	1.25	2.5	5	0.6	1.25	2.5	5
Mg/kg/d	231	481	975	1982	268	553	1121	2311
HED (m/k/d)	38.5	80.1	162.5	330.7	44.7	92.1	186.8	385.1

Results:

Body weights and food consumptions

⁵ Kaufmann et al., Nutrition 24: 462-469, 2008

⁶ Kaufmann et al., JPEN 27(6): 411-418, 2003

⁷ Little 1953 a cited from IPCS INCHEM 1988; Ebert, Toxicol Letters 3: 65-70, 1979a; Ebert, Toxicol Letters 3: 771-78, 1979b

At 5.0% MSG, significant decreases in body weight gains or trends were observed in males at Week 98 and in females at Week 90 and remained so until the end of the study; however, there was no decrease in food consumption in either sex.

Histopathological findings

Previously, findings of epithelial hyperplasia in renal pelvis and urinary bladder of dogs administered 6% MSG in a cereal diet for 13 weeks were reported. The authors examined the effect of MSG on the urinary tract closely. Data for non-neoplastic, proliferative, and neoplastic lesions are provided in the table below.

Table 11: Incidences of non-neoplastic, proliferative, and neoplastic lesions in the urinary tract

(Table from the article)

Urinary tract/ finding	MSG (%) in diet of males					MSG (%) in diet of females				
	0 (50)	0.6 (50)	1.25 (49)	2.5 (50)	5.0 (50)	0 (50)	0.6 (50)	1.25 (50)	2.5 (50)	5.0 (49)*
Kidneys										
Chronic nephropathy (+)	9	4	7	10	6	2	5	4	2	5
(++)	2	6	7	6	4	1	0	1	4	1
(+++)	0	3	2	0	1	0	1	0	1	0
Interstitial nephritis	0	1	0	2	0	0	0	1	1	0
Pyelonephritis	0	0	0	0	0	0	0	0	0	0
Hydronephrosis	0	1	0	0	0	0	0	0	0	0
Tubular cell hyperplasia	0	1	1	0	0	0	0	0	2	0
Transitional cell hyperplasia, pelvis	0	1	4	1	4	4	1	3	1	1
TCC†, pelvis	0	0	0	1	0	0	0	0	0	0
Nephroblastoma	0	0	0	0	0	2	2	1	0	0
Urinary bladder										
Diffuse papillary hyperplasia	0	0	1	0	1	3	0	0	2	1
Simple hyperplasia	0	0	0	1	0	1	0	0	0	1
PN hyperplasia‡	0	0	1	0	1	0	0	0	1	0
Papilloma	0	1	0	1	0	3	0	0	2	0
TCC†	0	0	0	0	0	1	0	0	2	0

*Number of rats in parentheses.

†TCC, transitional cell carcinoma.

‡PN, papillary or nodular.

Summary of non-neoplastic findings:

- Transitional cell hyperplasia of the renal pelvis

The findings were not dose dependent. An increased incidence was noted in male rats (1.25% and 5% MSG) and females (control and 1.25% MSG). The finding was often associated with chronic nephropathy or renal inflammatory lesions.

- Urinary bladder:

Diffuse papillary hyperplasia (papillomatosis) was associated with presence of calculi.

Summary of proliferative and neoplastic lesions:

There was no increase in incidence of proliferative and neoplastic lesions in the kidneys or urinary bladder. A variety of neoplastic lesions were observed in various organs; however, they were at similar incidence in control and MSG-treated rats of both sexes (data not shown).

Human experience

In humans, results from several studies in which healthy adult male and female subjects received oral supplemental L-glutamine at daily doses ranging from 3-45 g for up to 10 weeks did not reveal any compound-related adverse effects (Candow et al., 2001; Falk et al., 2003; Kerksick et al., 2006; Krieger et al., 2004; Lehmkuhl et al., 2003; Rathmacher et al., 2004; Thistlethwaite et al., 2005; Williams et al., 2002; Ziegler et al., 1990). In a recent review of the safety of L-glutamine, Shao and Hathcock (2008) concluded that there is strong evidence in humans supporting an absence of adverse effects associated with the supplemental consumption of up to 14 g L-glutamine/day.

Update labeling in Section 13.1

L-glutamine was not mutagenic in a bacterial mutagenicity (Ames) assay, nor clastogenic in a chromosomal aberration assay in mammalian (Chinese Hamster Lung CHL/IU) cells.

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/s/

SHWU LUAN LEE
06/06/2017

CHRISTOPHER M SHETH
06/06/2017

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 208587
Supporting document/s: 1
Applicant's letter date: September 7, 2016
CDER stamp date: September 7, 2016
Product: Endari (L-glutamine)
Indication: Treatment for sickle cell disease
Applicant: Emmaus Medical, Inc.
Review Division: Division of Hematology Oncology Toxicology
(DHOT) for Division of Hematology Products
(DHP)
Reviewer: Shwu-Luan Lee, PhD
Supervisor/Team Leader: Christopher Sheth, PhD
Division Director: John Leighton, PhD
Project Manager: Michael Gwathmey, RN

Disclaimer

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	5
1.1	INTRODUCTION	5
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	5
1.3	RECOMMENDATIONS	5
2	DRUG INFORMATION	6
2.1	DRUG.....	6
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs	6
2.3	DRUG FORMULATION	6
2.4	COMMENTS ON NOVEL EXCIPIENTS.....	6
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	6
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	9
2.7	REGULATORY BACKGROUND	9
3	STUDIES SUBMITTED	9
3.1	STUDIES REVIEWED.....	9
3.2	STUDIES NOT REVIEWED	9
3.3	PREVIOUS REVIEWS REFERENCED	9
4	PHARMACOLOGY	9
4.1	PRIMARY PHARMACOLOGY	10
4.2	SECONDARY PHARMACOLOGY	19
4.3	SAFETY PHARMACOLOGY	19
5	PHARMACOKINETICS/ADME/TOXICOKINETICS.....	20
5.1	PK/ADME	20
5.2	TOXICOKINETICS.....	20
6	GENERAL TOXICOLOGY.....	20
7	GENETIC TOXICOLOGY.....	21
8	CARCINOGENICITY	22
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	22
10	SPECIAL TOXICOLOGY STUDIES.....	24
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	24
12	APPENDIX/ATTACHMENTS.....	24

Table of Tables

Table 1: The summary of the daily intake of heavy metal impurities	7
Table 2: Batch comparison.....	8
Table 3: In-use Reconstitution Stability Study Results (NutreStore® Product).....	9
Table 4: Blood parameters from samples from normal volunteers, and patients with SCD and autoimmune hemolytic anemia	12
Table 5: Effect of manipulating the NAD ⁺ /NAD ^r ratio on the RBC DPG content in vitro	13
Table 6: Glutamine transport in RBCs from three groups of samples	15
Table 7: Glutamine concentrations and Km for active glutamine transport (ex vivo data)	15
Table 8: NADH, total NAD, redox potential, and hemoglobin at baseline and after 4 weeks of L-glutamine administration	16
Table 9: Ratios of HUVEC adhesion rates of sickle RBC to those of normal RBC	17
Table 10: Summary of findings in the toxicology studies	20
Table 11: Influence of amino acid on SCE frequency in human lymphocytes	22
Table 12: Embryotoxic activity of 3-aminoglutaramide and L- and DL-glutamine	23

Table of Figures

Figure 1: Chemical structure of L-glutamine	6
Figure 2: NAD metabolism in the human erythrocyte	14
Figure 3: Ratios of HUVEC adhesion rates of sickle RBC to those of normal RBC before and after treatment of oral L-glutamine (30 g/day)	18

1 Executive Summary

1.1 Introduction

This review supports the use of orally administered L-glutamine (an amino acid) for the treatment of sickle cell disease (SCD). The nonclinical information in this application is derived entirely from pharmacodynamic, pharmacokinetic (PK), and toxicology data in the scientific literature and additional toxicology studies provided by the L-glutamine supplier [REDACTED] (b) (4). Other pharmacology/toxicology data used to support this 505(b)(2) NDA were previously reviewed under NDA 21667. The data reviewed under NDA 21667 were reviewed by Dr. Ke Zhang, and are referred to in the respective sections in this NDA review. Emmaus conducted several primary pharmacology studies in humans to support additions to Section 12.2 of the label (i.e., Pharmacodynamics), and are not discussed in the current review.

1.2 Brief Discussion of Nonclinical Findings

The pharmacology data provided comes mainly from published scientific articles, and provides the rationale for using L-glutamine in the treatment of SCD and related complications of the disease, such as severe anemia, frequent vasoocclusive processes, and occlusion which damages tissues.

Increases in the rate of glutamine transport and higher active red cell glutamine affinity may increase glutamine availability, and increase the total nicotinamide adenine dinucleotide (NAD) content in sickle red blood cells (RBCs). Research data suggests that sickle RBCs have decreased NAD redox potential, manifested as decreased $\text{NADH}/[\text{NAD}^+ + \text{NADH}]$ ratios, when compared with normal RBCs. At the same time, sickle RBCs have higher total NAD content than normal RBCs. Decreased NAD redox potential renders sickle RBCs more susceptible to oxidative damage. Oxidative damage may result in stimulation of inflammatory processes and expression of adhesion molecules in RBCs. Thus L-glutamine may improve the condition of sickle RBCs by increasing the NAD redox potential and NADH levels, thereby preventing some of the oxidative damage they typically experience.

Most of the safety toxicology studies for L-glutamine that support this NDA (205857) were reviewed under NDA 21667. The current review cites excerpts of the review for NDA 21667 where relevant. In brief, the oral LD_{50} values were approximately 20 g/kg in mice and rabbits and approximately 10 g/kg in rats. The potential targets of organs of toxicity include the stomach, liver, and kidney.

1.3 Recommendations

1.3.1 Approvability

L-glutamine is approvable for the proposed indication from the perspective of Pharmacology/Toxicology.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The label for this product will retain much of the labeling for the Listed Drug (NutreStore) with respect to the pharmacology/toxicology sections, with minor modifications to support the currently proposed indication.

2 Drug Information

2.1 Drug

The current 505(b)(2) NDA relies on data and references filed with the Listed Drug. According to the Applicant, the drug substance and drug product for this application are manufactured by the same manufacturers (b) (4), respectively), per the same manufacturing processes, as those used for the Listed Drug under NDA 21667. See the CMC review for more detailed information.

CAS Registry Number	56-85-9
Generic Name	L-glutamine
Code Name	N/A
Chemical Name	(S)-2-aminoglutaramic acid, L-glutamic acid 5-amide, (S)-2,5-diamino-5-oxopentanoic acid
Molecular Formula/Molecular Weight	C ₅ H ₁₀ N ₂ O ₃ /146.15 Da
Structure or Biochemical Description	<p>Figure 1: Chemical structure of L-glutamine</p> <chem>NC(=O)CC(N)C(=O)O</chem>
Pharmacologic Class	Amino acid

2.2 Relevant INDs, NDAs, BLAs and DMFs

Listed drug: NutreStore, NDA 21667 (Emmaus Medical, Inc.)

DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4)

2.3 Drug Formulation

The drug product consists of a sealed packet containing 5 grams of L-glutamine powder.

2.4 Comments on Novel Excipients

No excipients are added and no additional processing is performed; besides packaging of the bulk L-glutamine into the unit dose packets.

2.5 Comments on Impurities/Degradants of Concern

The nonclinical studies on L-glutamine derived from the scientific literature have methods of analysis that vary by study. The identity and quantity of the impurities and degradants in the drug substances used in these studies is not available to Emmaus.

The toxicology studies conducted by (b) (4) were not managed under Good Laboratory Practices because they were conducted before 1979.

The proposed drug substance specifications are based on those filed under NDA 21667. Changes from the previous specifications have been made to account for the more conservative limits for elemental impurities in drug products that currently exist.

The Applicant previously elevated the specification of impurity (b) (4). The Pharmacology/Toxicology Reviewer from DGIEP, Dr. Tamal K. Chakraborti, performed a safety assessment and accepted the increased specification. See Appendix for more information.

The elemental impurities (i.e., heavy metal impurities) (b) (4)

Table 1: The summary of the daily intake of heavy metal impurities

	(b) (4)
Specification	(b) (4)
Daily intake at 18 g/day*	(b) (4)
At 30 g/day*	(b) (4)
PDE (ICH Q3D)	(b) (4)

Although the contents of (b) (4) in the three clinical lots are much lower than the proposed specifications for these impurities (see table below), at the maximum oral dose of 30 g/day and the proposed specification, the intakes would nominally exceed the oral PDEs listed in the Q3D guidance by (b) (4) µg/day.

Table 2: Batch comparison

Table from the Applicant

(b) (4)

Drug product

- Specifications

The specifications of related substances (degradants and impurities) filed for this application are the same as those for NDA 21667 and are acceptably safe (see above).

- Stability in water

A reconstitution study was performed by (b) (4) to determine the stability of the L-glutamine product when dissolved in water. Glutamine content did not decrease with time, and the only related impurity that increased with time was (b) (4). (b) (4) content increased by less than (b) (4) % over six hours. These data are consistent with the in-use study results (see Table below).

Table 3: In-use Reconstitution Stability Study Results (NutreStore® Product)

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

SCD: The proposed dose of L-glutamine powder for oral solution is 0.3 g/kg of L-glutamine in increments of 5 g based on weight, with an upper limit of 30 g/day, administered orally twice daily. 0.3 g/kg (or 18 g/day for a 60 kg person)

2.7 Regulatory Background

L-glutamine has been well-studied under a number of clinical conditions and is currently approved for the treatment of Short Bowel Syndrome (SBS) in patients receiving specialized nutritional support when used in conjunction with a recombinant human growth hormone (rhGH) (NDA 21667, Emmaus).

3 Studies Submitted

3.1 Studies Reviewed

Study titles and study numbers are included in the relevant sections.

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

NDA 21667

4 Pharmacology

SCD is a group of inheritable blood disorders associated with abnormal hemoglobin, called hemoglobin S or sickle hemoglobin. When a person inherits two hemoglobin S genes, or hemoglobin SS, the disease is called sickle cell anemia (SCA). The abnormal

hemoglobin S results in diminished capacity for RBCs to carry oxygen. It can also lead to rigid sickle shaped RBCs, for which the disease is named.

Sickle RBCs can't change shape easily, so they tend to burst apart or hemolyze. Normal RBCs have a lifespan of about 90 to 120 days in circulation, whereas sickle RBCs last 10 to 20 days. The body is always making new RBCs via hematopoiesis to replace the old cells; however, in SCD the body may have trouble keeping up with how fast the cells are being destroyed. Because of this, the number of RBCs is usually lower than normal.¹ Sickle-shaped RBCs are not flexible and can stick to vessel walls, causing a blockage that slows or stops the flow of blood. When this happens, oxygen cannot reach nearby tissues. The lack of tissue oxygen can cause attacks of sudden, severe pain, called pain crises. The red cell sickling and poor oxygen delivery can also cause organ damage.

Complications of SCD are usually due to severe anemia and frequent vasoocclusive processes which damage tissues. The cause of these events is attributed largely to increased adherence of sickle RBCs to vascular endothelium.²

Modalities used in treatment of people with SCD may include preventing infections with vaccinations and antibiotics, high fluid intake, folic acid supplementation, and pain medication. Other measures may include blood transfusions, and administration of hydroxyurea. Bone marrow transplantation is curative in a small subset of patients.

4.1 Primary Pharmacology

Mechanism of action

L-glutamine is an essential amino acid found in abundance in the human body. L-glutamine's biological roles include: 1) a precursor of nucleic acids and nucleotides such as the pyridine nucleotides, NAD and its reduced form NADH, 2) a preferred fuel for rapidly dividing cells including hematopoietic cells, and 3) a precursor for glutathione (GSH).

The Applicant proposes that the rationale for using L-glutamine to treat patients with SCD resides mainly with the ability of L-glutamine to increase the activity of NAD synthesis and elevate the NAD redox potential in sickle RBCs to counter the oxidant-dependent pathophysiology of the disease.

Erythrocyte NAD redox potential in SCD

Article title: Decreased erythrocyte nicotinamide adenine dinucleotide redox potential and abnormal pyridine nucleotide content in sickle cell disease (Zerez et al., Blood 71: 512-515, 1988)

¹ Wikipedia "Sickle-cell disease": https://en.wikipedia.org/wiki/Sickle-cell_disease

² Frenette PS, Curr Opin Hematol 9: 101-106, 2002

Key findings:

Sickle RBCs have a significant decrease in the $\text{NADH}/(\text{NAD}^+ + \text{NADH})$ ratio (i.e., NAD redox potential) compared with normal RBCs.

The key antioxidant defense reactions are believed to be linked to the pyridine nucleotides, i.e., NAD and nicotinamide adenine dinucleotide phosphate ([NADP]). The RBC redox potential is controlled by the ratio of NADH to $[\text{NAD}^+ + \text{NADH}]$ as well as the NADPH to $[\text{NADP}^+ + \text{NADPH}]$ ratios. Using blood samples obtained from normal subjects, individuals with reticulocytosis, and individuals with SCD, the authors demonstrated that sickle RBCs have a significant decrease in the $\text{NADH}/[\text{NAD}^+ + \text{NADH}]$ ratio compared with normal RBCs, despite a significant increase in total NAD content, when compared to normal RBCs. In sickle RBCs, however, the NADPH to $[\text{NADP}^+ + \text{NADPH}]$ ratio was not significantly altered. The authors concluded that sickle RBCs have a decrease in NAD redox potential that may be a reflection of their increased oxidant sensitivity. The changes in these pyridine nucleotides may have further metabolic consequences for the sickle erythrocyte.

Article title: Relationship between the nicotinamide adenine dinucleotide (NAD) redox potential and the 2,3-diphosphoglycerate content in the erythrocyte in sickle cell disease (SCD) (Lachant et al., British Journal of Hematology 72: 265-271, 1989)

Key finding:

Sickle cells have a decrease in NAD redox potential manifested by a lower ratio of NADH to total NAD (i.e., NADH plus NAD^+) and a compensatory increase in total NAD concentration. Oxidant damage in SCD may be related to an impairment of antioxidant defense mechanisms.

Previously, the role of oxidative stress in the pathogenesis of SCD was investigated by the authors.³ In sickle RBCs, there was a significant increase in incubated Heinz body formation, a decrease in GSH concentrations, an increase in glucose-6-phosphate dehydrogenase (G6PD) activity, and a decrease in GSH reductase activity compared to RBCs from individuals with reticulocytosis. Their study results suggest that in spite of an absolute increase in stimulated pentose shunt (PPS) activity, there is a relative suppression of stimulated shunt activity in the youngest sickle erythrocytes. This may be related, in part, to the inhibitory effects of high concentrations of ATP on the activity of glucose-6-phosphate dehydrogenase. Two antioxidants, ascorbic acid and α -mercaptopyropionylglycine, were found to reduce Heinz body formation during incubation with acetylphenylhydrazine. Ascorbic acid protected the sickle RBC against hydrogen peroxide induced hemolysis and increased the rate of glucose oxidation by the PPS.⁴

It is well documented that the $\text{NAD}^+/\text{NAD}_r$ ratio⁵ is increased in the RBC in SCD. The authors hypothesized that the increased $\text{NAD}^+/\text{NAD}_r$ ratio resulted from the increased 2,3-diphosphoglycerate (DPG) content of the SCD RBC. Previous investigations

³ Lachant et al., Am J Hematol 15: 1-13, 1983

⁴ Lachant and Tanaka, Am J Med Sci 292: 3-10, 1986

⁵ $\text{NAD}^+/\text{NAD}_r$ ratio: percentage of NAD in the oxidized form $[\text{NAD}^+ / (\text{NAD}^+ \text{ and } \text{NADH})]$

suggested that an increase in the NAD^+ concentration should increase the glycolytic flux through glyceraldehyde-3-phosphate dehydrogenase (NAD oxidoreductase, G3PD) and secondarily increases the DPG content of the RBC.

An inverse relationship was observed between the packed cell volume (PCV) and the RBC DPG concentration ($r = -0.69$), and a direct relationship was observed between the RBC $\text{NAD}^+/\text{NAD}_T$ ratio and the DPG content for the entire study population ($r = 0.74$).

Table 4: Blood parameters from samples from normal volunteers, and patients with SCD and autoimmune hemolytic anemia

Table from the article

	I Normal (n = 13)	P value I v II	II SCD (n = 11)	P value II v III	III AIHA (n = 3)
Packed cell volume (l/l)	0.43 ± 0.04	<0.001	0.26 ± 0.04	NS	0.27 ± 0.09
Reticulocytes (%)	1.1 ± 0.5	<0.001	27.4 ± 14.0	NS	42.1 ± 32.5
DPG (mmol/l)	4.92 ± 0.51	<0.001	6.38 ± 0.72	NS	6.13 ± 0.72
$\text{DPG}_{\text{adj(PCV)}} \text{ (mmol/l)}$	5.44 ± 0.51	<0.07	5.95 ± 0.74	NS	5.67 ± 0.76
$\text{DPG}_{\text{adj(NAD)}} \text{ (mmol/l)}$	5.30 ± 0.54	<0.02	5.85 ± 0.50	<0.07	6.46 ± 0.22
$\text{NAD}^+/\text{NAD}_T$	0.49 ± 0.10	<0.001	0.64 ± 0.12	<0.07	0.53 ± 0.15

NS = not significant.

Table 5: Effect of manipulating the NAD⁺/NAD_T ratio on the RBC DPG content in vitro

(Table from the article)

Additive	RBC	NAD ⁺ /NAD _T		n	DPG (mmol/l)		P value*
		0 min	180 min		0 min	180 min	
None	NL	0.456	0.425	5	4.58±0.53	NS	4.23±0.18
	AIHA	—	—	1	5.00	—	5.00
	SCD	—	—	1	5.75	—	5.93
Acetylphenylhydrazine	NL	0.446	0.575	3	4.97±0.48	<0.05	6.22±0.96
New methylene blue	NL	0.473	0.551	5	4.43±0.55	<0.02	4.92±0.72
	AIHA	—	—	1	5.15	—	5.48
'Lactate trap'	NL	0.404	0.719	4	5.04±0.59	<0.04	5.70±0.48
	AIHA	—	—	1	4.90	—	5.50
'Pyruvate trap'	NL	0.473	0.269	5	4.78±0.59	<0.02	4.45±0.70
	AIHA	—	—	1	5.10	—	4.64
	SCD	—	—	1	5.57	—	4.18

n = Number; NL = Normal; SCD = Sickle cell disease; AIHA = Autoimmune haemolytic anaemia; NS = Not significant.

* Paired t-test (0 v 180 min).

Effects of L-glutamine on the erythrocyte in SCD

Article title: Increased red cell glutamine availability in sickle cell anemia: Demonstration of increased active transport, affinity, and increased glutamate level in intact red cells (Niihara et al., J Lab Clin Med 130 (1): 83-90, 1997)

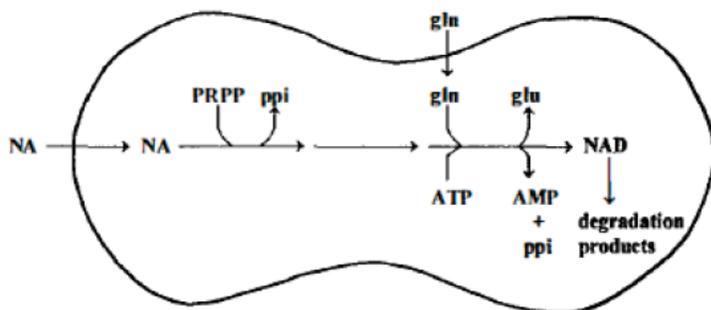
Key finding:

- There is an increased utilization of glutamine by intact sickle RBCs in conjunction with NAD metabolism in vitro.
- There was a significant increase in the rate of transport for L-glutamine.
- L-glutamine availability may play a role in the increased NAD in sickle RBCs.

The mechanism of an increased NAD content in sickle RBCs was investigated. The NAD metabolism in the RBC is depicted in the figure below:

Figure 2: NAD metabolism in the human erythrocyte

Figure from the article



NA, Nicotinic acid; PRPP, 5-phosphoribosyl-1-pyrophosphate; ppi, pyrophosphate; gln, glutamine; glu, glutamate; ATP, adenosine triphosphate; AMP, adenosine monophosphate

Previously, the authors found that there was no significant change in the rate of nicotinic acid transport in sickle cells as compared with that in high-reticulocyte control subjects. There was a slight decrease in NAD degradation, but it was not sufficient to explain the degree of increase in total NAD. Because L-glutamine is an essential precursor in NADH biosynthesis, the following parameters were determined to investigate the role of L-glutamine in increasing NAD in sickle RBCs: rate of active RBC glutamine transport, and the kinetics of the transport expressed with Michaelis-Menten constant (K_m) and maximum velocity (V_{max}). In addition, plasma and RBC levels of glutamine and glutamate (a by-product of glutamine in NAD synthesis) were also measured. The study was conducted using RBCs from patients with SCD, and RBCs from patients with high reticulocyte counts, and normal volunteers as controls.

Intra-erythrocytic levels of glutamine and glutamate were calculated by using the following formula:

$$C_{bc} = (C_{wb} - C_p[1 - Hct]) \div Hct,$$

where C_{bc} = intracellular concentration, C_{wb} = whole blood concentration, C_p = plasma concentration, and Hct = hematocrit.

The result indicated that glutamine transport rate was increased; however, intracellular L-glutamine concentration was not elevated. Instead, the level of glutamate, a byproduct of glutamine in NAD synthesis, was increased in sickle RBC in comparison to high reticulocyte controls.

Table 6: Glutamine transport in RBCs from three groups of samples

Table from the article

Sample type	Number of patients	Reticulocyte count (%)	Hexokinase ($\mu\text{mol}/\text{min} \cdot 10^{10}$ RBCs)	Glutamine transport rate Na KRP – choline KRP (nmol gln/min · ml RBC)
Normal				
Mean \pm 1 SD	7	1.6 \pm 0.3	0.29 \pm 0.07	1.8 \pm 0.78
Range		(1.2-2.0)	(0.20-0.42)	(1.2-3.2)
High reticulocyte				
Mean \pm 1 SD	6	9.0 \pm 7.8	0.82 \pm 0.16	10.8 \pm 9.6
Range		(4.2-24.4)	(0.60-1.07)	(3.5-29.3)
Hb SS				
Mean \pm 1 SD	11	11.4 \pm 4.2	0.97 \pm 0.33	31.0 \pm 15.3
Range		(5.7-18.6)	(0.51-1.76)	(10.1-53.6)

⁷ test: Reticulocyte count: normal versus high reticulocyte, $p < 0.05$; normal versus Hb SS, $p < 0.00005$; HR versus Hb SS, not significant. Hexokinase: normal versus high reticulocyte, $p < 0.00005$; normal versus Hb SS, $p < 0.0001$; HR versus Hb SS, not significant. Glutamine transport rate: normal versus HR, $p < 0.05$; normal versus Hb SS, $p < 0.0002$; HR versus Hb SS, $p < 0.02$.

When the rate of glutamine transport is examined as a function of cell age, by measuring either the reticulocyte count or the hexokinase activity, there appears to be an indirectly proportional linear relationship with cell age. Based on these readouts, glutamine transport rate appears to be higher in sickle cells than in cells from high-reticulocyte control subjects. A decreased K_m and increased V_{max} for glutamine transport in sickle RBC was noted.

Glutamine transport kinetics, K_m

Normal individuals	410 \pm 165 $\mu\text{mol}/\text{L}$
High-reticulocyte control subjects	360 \pm 203 $\mu\text{mol}/\text{L}$
Patients with SCD	102 \pm 57 $\mu\text{mol}/\text{L}$

Reviewer's note:

The K_m values were comparable with the result of Zerez 1994 (see below).

Article title: Increased red-cell glutamine availability in sickle-anemia. 2. Evidence for higher affinity red-cell glutamine transport and higher plasma glutamine concentration (Zerez et al., Blood 84: 10000411-10000411, 1994)

Sickle cells were shown to have a several fold increase in the rate of glutamine transport compared to cells of equivalent cell age. In this abstract; the authors measured plasma and red cell glutamine levels and examined kinetic aspects of glutamine transport (the mean \pm SD is shown). The results are tabulated as follows.

Table 7: Glutamine concentrations and K_m for active glutamine transport (ex vivo data)

Blood samples*	[Glu] Fasting plasma (μM)	[Glu] RBC (μM)	K_m (μM)
Normal	625 \pm 98	645 \pm 142	509 \pm 69
High reticulocytes	505 \pm 100	743 \pm 148	162 \pm 53

SCA	684 ± 128 ^a	890 ± 223 ^{b,c}	52 ± 26 ^{b,c}
-----	------------------------	--------------------------	------------------------

*Blood samples were collected from normal individuals, patients with high reticulocytes and patients with SCA; [Glu]: glutamine concentration (μM)

a: Statistically significantly different between SC anemia and high reticulocytes

b, c: Statistically significantly different between SC anemia and high reticulocytes, SC anemia and normal individuals, respectively.

The combination of a higher plasma glutamine concentration and a higher affinity active red cell glutamine transport system support the hypothesis of increased red cell glutamine availability in SCA. Increased glutamine availability may be associated with increased total NAD content in sickle RBCs.

Article title: Oral L-glutamine therapy for sickle cell anemia: I. subjective clinical improvement and favorable changes in red cell NAD redox potential (Niihara et al., Am J Hematol 58: 117-121, 1998)

Key finding:

Oral L-glutamine can significantly increase the NAD redox potential and NADH level in sickle RBC. These changes may decrease oxidative susceptibility of sickle RBC and result in clinical benefit.

Seven patients with SCD, who were hydroxyurea treatment naïve or had no transfusions within 3 months of initiation of the study, received oral L-glutamine at 30 g/day for 4 weeks. Blood samples were drawn from the patients for evaluation of RBC total NAD and NADH levels.

Table 8: NADH, total NAD, redox potential, and hemoglobin at baseline and after 4 weeks of L-glutamine administration

(Table from the article)

	Baseline (N = 7)	Week 4 (N = 7)	P
NADH (nmol/ml RBC)	47.5 ± 6.3 (41.2–57.0)	72.1 ± 15.1 (52.4–96.0)	<0.01
Total NAD (nmol/ml RBC)	101.2 ± 16.0 (77.7–118.0)	116.4 ± 14.7 (98.3–132.1)	n/s
Redox potential (%)	47.2 ± 3.7 (42.7–54.1)	62.1 ± 11.8 (48.4–80.7)	<0.01
Hemoglobin (g/dL)	8.5 ± 1.2 (7.1–10.6)	8.7 ± 1.2 (7.1–10.7)	n/s

*Values in parentheses indicate range. NADH, nicotinamide adenine dinucleotide reduced; NAD, nicotinamide adenine dinucleotide; RBC, red blood cells.

In addition, among the seven patients, there was an improvement in subjective clinical responses, including increased energy level and activity level, and decreased chronic pain level and narcotics dosage.

Endothelial adhesion of sickle RBCs

In SCA, inflammatory (i.e., intravascular sickling and transient vasoocclusive) events result in chronic endothelial activation. In addition to sickling behavior, sickle RBCs exhibit abnormal interaction with the vascular endothelium, which is considered to have an important role in initiation of vasoocclusion. Possible mechanisms underlying increased sickle RBC adhesion to the endothelium include:

- Oxidants and cytokines induced upregulation of endothelial adhesion molecules in the sickle RBC; these adhesion molecules include CD36, $\alpha_4\beta_1$ -integrin, intercellular adhesion molecule-4 (ICAM-4), and basal cell adhesion molecule.
- Activated endothelium: Increased P-selectin, vascular cell adhesion molecule-1 (VCAM-1), and $\alpha_v\beta_3$ -integrin.
- Involvement of plasma factors and adhesive proteins, such as thrombospondin, von Willebrand factor (vWF), and laminin.

Supplemental glutamine may increase the activity of NAD synthesis, thereby countering the oxidant-dependent pathophysiology of sickle RBC.

Article title: L-Glutamine therapy reduces endothelial adhesion of sickle red blood cells to human umbilical vein endothelial cells (Niihara et al., BMC Blood Disorders 5: 4, 1-7, 2005)

Key finding:

Oral L-glutamine administration consistently resulted in improvement of sickle RBC adhesion to human umbilical vein endothelial cells (HUVEC). These data suggest positive physiological effects of L-glutamine in SCD.

The effect of L-glutamine on adhesion of sickle RBC to human umbilical vein endothelial cells (HUVEC) was examined. Five adult patients with SCD (≥ 18 years old) were treated with oral L-glutamine 30 g/day for 4-8 weeks, while four adult patients served as the controls and received no treatment. Normal control samples were drawn from healthy volunteers. HUVEC were harvested from umbilical cord veins according to a conventional method and passaged every 4-5 days. The cells were used from passages 2 to 6.

Results

Table 9: Ratios of HUVEC adhesion rates of sickle RBC to those of normal RBC

	L-glutamine treated group	Un-treated group
HUVEC + autologous plasma	0.97 \pm 0.45 ^a	1.91 \pm 0.53
HUVEC + autologous plasma + LPS	1.39 \pm 0.33	2.80 \pm 0.47 ^b

a: statistically different compared to un-treated group ($p < 0.02$)

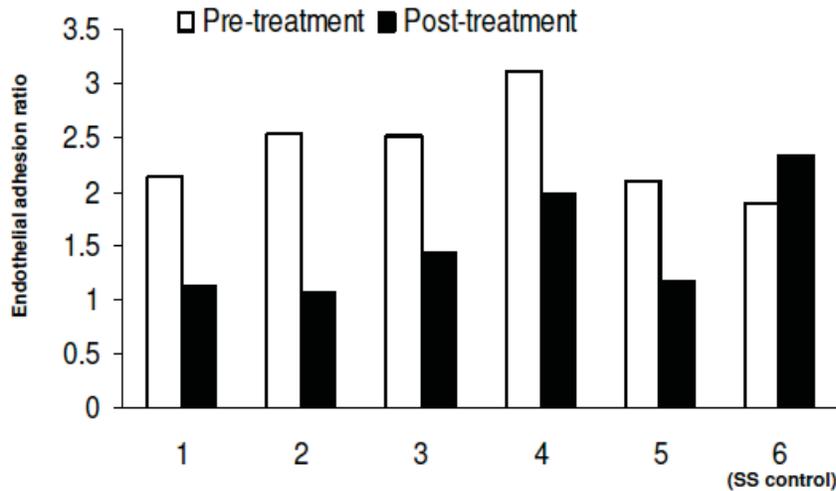
b: statistically different compared to un-treated group ($p < 0.001$)

LPS: lipopolysaccharide

Treatment of L-glutamine in the presence of LPS significantly reduced sickle RBC adherence to HUVEC, compared to the untreated group ($p < 0.001$).

Figure 3: Ratios of HUVEC adhesion rates of sickle RBC to those of normal RBC before and after treatment of oral L-glutamine (30 g/day)

(Figure from the article)



Treatment of L-glutamine for 4 to 8 weeks, reduced RBC adherence to HUVEC (mean decrease of the adhesion ratios: 1.13 ± 0.21 with $p < 0.001$), but did not change reticulocyte counts or hemoglobin levels in five patients with SCA. For the patient who participated as sickle cell control, the sickle RBC adhesion to endothelial cells increased slightly compared to the baseline.

Discussion

The exact mechanism by which L-glutamine effectively decreases adhesion of sickle RBCs to the endothelium is not clear. The authors hypothesized that the mechanism includes L-glutamine-related improvement of the NAD redox potential of SCD RBCs. Oxidant damage may result in stimulation of inflammation and expression of adhesion molecules in RBC. L-glutamine may also provide energy and building materials that could help maintain the integrity of sickle RBCs. Anecdotal reports suggest that children with sickle cell disease require increased amino acid consumption, especially glutamine.⁶

Article title: Inhibition of sickle red cell adhesion and vasoocclusion in the microcirculation by antioxidants (Kaul et al., *Am J Physiol Heart Circ Physiol* 291: H167-H175, 2006)

Key finding:

- Inflammatory activation of vascular endothelium as a consequence of increased oxidant generation increases sickle red cell adhesion.

⁶ Salman et al., *Pediatr Res* 40: 34-40, 1996.

- Antioxidant enzymes (superoxide dismutase [SOD] and catalase) and polynitroxyl albumin (PNA) have inhibitory effects on sickle red cell adhesion in a platelet-activating factor (PAF)-activated ex vivo preparation.

The authors investigated the effect of antioxidants (SOD, catalase and SOD mimetic, polynitroxyl albumin, PNA), in the presence of platelet-activating factor (PAF), on sickle RBC on endothelium adhesion. PAF is documented to cause endothelial oxidant generation in sickle RBC and endothelial activation and the resultant sickle RBC adhesion and blockage of small diameter venules observed in SCA. Furthermore, PNA, an effective antioxidant, was reported to abolish sickle red cell adhesion. Thus sickle red cell adhesion and related vasoocclusion may be ameliorated by antioxidant therapy.

The authors investigated the relationship between endothelial activation and sickle RBC adhesion to endothelium. An ex vivo mesoecum vasculature model was used that allows measurement of hemodynamic parameters (such as peripheral resistance) and intravital microscopy for quantification of adhesion. The inhibitory effect of antioxidants on adhesion was assessed. The ex vivo preparation was stimulated by PAF to induce inflammatory effects mimicking characteristics of SCD, including endothelial oxidant generation, endothelial activation, and microvascular injury. PAF is elevated twofold in patients with sickle cell disease,⁷ suggesting that PAF has a role in chronic endothelial activation and inflammation in this disease.

The result demonstrated that sickle RBCs adhesion was enhanced by activation of endothelial inflammation. Antioxidant agents were shown to inhibit the adhesion of sickle RBCs to the endothelium. L-glutamine increases GSH levels and acts as an antioxidant, improving sickle red cell NAD redox potential. Oral L-glutamine administration resulted in improvement of sickle RBC adhesion to HUVEC (see above).

L-glutamine and GSH

L-glutamine is the precursor of GSH. GSH supplementation helps prevent deficiencies of glutamine and GSH and improves protein metabolism in tumor-bearing rats. (Journal of Parenteral and Enteral Nutrition 19:492-497, 1995)

4.2 Secondary Pharmacology

No studies submitted in this NDA.

4.3 Safety Pharmacology

No studies submitted in this NDA.

⁷ Oh et al., J Lab Clin Med 130: 191-195, 1997

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The Applicant did not conduct any PK studies of L-glutamine in support of the marketing application for the treatment of SCD. The following statement is excerpted from Dr. Ke Zhang's summary (NDA 21667).

Glutamine level in the body is maintained by dietary intake and synthesis from endogenous glutamate. The typical daily dietary intake of glutamine is ~5-10 g. Oral absorption of glutamine was demonstrated with peak plasma level of glutamine reached at ~1-1.5 hours after dosing in rats. Glutamine was distributed in the liver, lung, kidney, heart, spleen, muscle, and brain following dietary or intravenous administration in rats. Glutamine is formed in the body through the condensation of a glutamate and an ammonia molecule by glutamine synthetase with hydrolysis of ATP. In the reverse process, glutaminase deaminated glutamine to glutamate and ammonia. Approximately 66% radioactivity (15N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

5.2 Toxicokinetics

No TK analyses were conducted.

6 General Toxicology

The table below is the summary of toxicology studies reviewed by Dr. Ke Zhang (NDA 21667)

Table 10: Summary of findings in the toxicology studies

Species	Study details	Key Findings/comments
Mice, rats and rabbits	Single maximum tolerated dose (MTD) [REDACTED] ^{(b) (4)} 1974; non-GLP)	LD ₅₀ values (g/kg) <ul style="list-style-type: none"> • Mouse: oral (20-22); IV (4.5) • Rat: oral (7.5-10.5) • Rabbit (male): oral (18.8)
Rats	Oral gavage ⁸ : 4, 6 ⁹ and 10 g/kg/day for 30 days (n=10/sex/group)	<ul style="list-style-type: none"> • Mortality: Glutamine was lethal in the high dose due to "administration of the test substance suspension in large volumes" ("physical problem"). • Mean body weight: ~7% lower in the high dose males as compared to the control • Catarrh¹⁰ in the stomach was noted in the L-glutamine groups but not in the control

⁸ L-glutamine was suspended in 5% aqueous gum Arabic solution at 25-50% and volume of 0.16-2 ml/100 g body weight was given by oral gavage.

⁹ It is noticed that the data from glutamine dose group of 6 g/kg/day were not presented in the tables in the result section.

		group.
Rats	Oral gavage: 2 and 4 g/kg/day for 180 days (n=10/sex/group) (non-GLP)	<ul style="list-style-type: none"> • Mean body weight: ~5% lower in the high dose females as compared to the control. • Slight decrease in hematocrit (10-11%) in the high dose males as compared to the control. • Serum glutamic pyruvic transaminase was significantly increased in the high dose males on Day 180 as compared to the control (26.8 IU and 12 IU, respectively). • Stomach: Catarrh in the stomach and infiltration of inflammatory cell and edema in gastric submucosa were identified in the both glutamine groups but not in control group. The catarrh in the stomach was noted on both Days 90 and 180. <p>On Day 180:</p> <ul style="list-style-type: none"> • Liver: fatty infiltration in the liver cells (slight, one each in the two dose groups) • Kidney: vacuolation of tubular epithelium (slight, 2 at high dose), protein case (slight, one at high dose)

7 Genetic Toxicology

The following is excerpted from NDA 21667 (Dr. Ke Zhang).

Study title: Amino Acid Excess Increase Sister-Chromatid Exchanges (SCEs) in Human Lymphocytes (Xing and Na, Mutation Research; 1996; 372:75-78).

The purpose of this study was to determine the effects of excessive amounts of amino acids including glutamine on SCEs in the cultures of human lymphocytes. The results indicated that addition of 10, 50, and 100 µg/ml of glutamine slightly increased the SCEs (11.05-11.50 SCEs/metaphase) as compared to the control (8.15 SCEs/metaphase) but this increase was not concentration dependent. There was no positive control in this study. The results were presented in Table 1 in this report. This table is attached below.

¹⁰ Excessive discharge or buildup of mucus in the nose or throat, associated with inflammation of the mucous membrane

Table 11: Influence of amino acid on SCE frequency in human lymphocytes

Table excerpted from Xin and Na, 1996

Amino acids (L-)	Original content ($\mu\text{g/ml}$) ^a	SCE/metaphase(mean \pm SE)					SCE ratios (mean)
		Control	Treatment ($\mu\text{g/ml}$ excess) ^b			Average	
			10	50	100		
Essential							1.61
Arginine	146	8.15 \pm 2.18	11.89 \pm 2.21	12.00 \pm 2.59	12.25 \pm 3.23	12.07 \pm 2.68	1.48
Cystine	37	8.15 \pm 2.18	14.00 \pm 2.35	13.85 \pm 2.82	14.25 \pm 2.95	14.03 \pm 2.71	1.73
Isoleucine	37	6.50 \pm 1.43	12.15 \pm 2.62	12.90 \pm 3.75	12.40 \pm 3.75	12.48 \pm 3.37	1.93
Leucine	37	6.50 \pm 1.43	9.61 \pm 1.85	10.05 \pm 2.50	10.40 \pm 3.62	10.02 \pm 2.66	1.52
Lysine	29	6.50 \pm 1.43	11.25 \pm 2.26	11.56 \pm 2.94	12.01 \pm 2.32	11.61 \pm 2.51	1.79
Methionine	11	6.50 \pm 1.43	9.10 \pm 2.65	9.65 \pm 2.43	9.75 \pm 2.69	9.50 \pm 2.59	1.45
Phenylalanine	11	6.62 \pm 2.20	7.85 \pm 1.95	8.00 \pm 2.10	8.21 \pm 2.22	8.02 \pm 2.09	1.21
Threonine	15	8.15 \pm 2.18	10.65 \pm 2.10	10.95 \pm 2.84	11.25 \pm 3.23	10.95 \pm 2.72	1.34
Tryptophan	4	6.62 \pm 2.20	8.81 \pm 2.24	9.27 \pm 2.88	9.55 \pm 2.23	9.21 \pm 2.45	1.38
Histidine	11	6.50 \pm 1.43	9.45 \pm 1.93	9.60 \pm 2.58	9.72 \pm 1.90	9.59 \pm 2.14	1.48
Tyrosine	15	8.15 \pm 2.18	13.44 \pm 3.22	13.14 \pm 2.38	13.25 \pm 2.69	13.28 \pm 2.76	1.63
Valine	15	6.50 \pm 1.43	10.25 \pm 1.94	10.37 \pm 2.72	10.65 \pm 2.45	10.42 \pm 2.37	1.61
Glutamine	219	8.15 \pm 2.18	11.24 \pm 1.89	11.50 \pm 2.20	11.05 \pm 2.28	11.26 \pm 2.12	1.40
Nonessential							1.49
Glutamic acid	15	6.62 \pm 2.20	8.95 \pm 1.32	9.30 \pm 2.29	9.85 \pm 2.35	9.37 \pm 1.97	1.43
Ornithine	-	6.62 \pm 2.20	10.25 \pm 2.49	10.14 \pm 3.38	10.15 \pm 2.72	10.18 \pm 2.86	1.57
Hydroxyproline	15	6.50 \pm 1.43	9.15 \pm 1.95	9.95 \pm 2.02	10.00 \pm 1.85	9.70 \pm 1.94	1.47
Proline	15	6.50 \pm 1.43	10.85 \pm 1.95	11.40 \pm 2.50	11.61 \pm 2.62	11.29 \pm 2.36	1.73
Alanine	-	6.50 \pm 1.43	10.15 \pm 2.66	10.15 \pm 2.92	10.20 \pm 2.24	10.11 \pm 2.61	1.56
Aspartic acid	15	6.50 \pm 1.43	9.15 \pm 1.45	9.45 \pm 2.32	9.71 \pm 2.76	9.26 \pm 2.18	1.45
Serine	22	6.50 \pm 1.43	9.65 \pm 2.45	9.30 \pm 2.75	10.05 \pm 2.84	9.67 \pm 2.68	1.52
Glycine	7	6.62 \pm 2.20	8.21 \pm 1.87	8.09 \pm 2.04	8.33 \pm 1.35	8.21 \pm 1.75	1.21

For all treatments, $p < 0.01$.^a Representing the original final concentrations of amino acids in culture medium calculated based on the original content of each amino acid in RPMI 1640.^b The final concentrations of the additional dose of each amino acid applied.

The small and concentration-independent increase may not be of any clinical significance.

8 Carcinogenicity

The Applicant submitted a Carcinogenicity Assessment Document (CAD) to support a request for waiver of the requirement to conduct a nonclinical carcinogenicity assessment for oral L-glutamine. The review of the CAD and any recommendations stemming from the review will be filed under a separate review.

9 Reproductive and Developmental Toxicology

(The following is excerpted from NDA 21667, Dr. Ke Zhang)

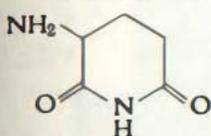
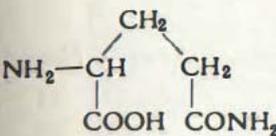
Study title: Studies on the relationship between the chemical structure and embryotoxic activity of thalidomine and related compounds (R.L. Smith, et.al., In: Robson JM, et. al., eds. Embryopathic Activity of Drugs. Ed. Boston: Little, Brown and Company; 1965:194-209).

Key study findings: Treatment with L-glutamine orally at 150 mg/kg/day during gestation days 7-12 in pregnant rabbits was not teratogenic. However, this study is not considered as a valid study since the treatment with glutamine during gestation days 7-12 did not cover the entire organogenesis of rabbits (gestation days 6-20 in rabbits).

The aim of the studies was to evaluate the potential embryotoxicity of 3-aminoglutarimide and related compounds including L-glutamine. In these studies, pregnant rabbits were given L-glutamine orally at 150 mg/kg/day during gestation days 7-12. The results indicated that treatment with L-glutamine was not teratogenic at this dose. However, this study is not considered as a valid study since the treatment with glutamine during gestation days 7-12 did not cover the entire organogenesis (gestation days 6-20). Also, the dose of L-glutamine tested (150 mg/kg/day) was not sufficiently high. The results were presented in Table 5 of this report (see Table below).

Table 12: Embryotoxic activity of 3-aminoglutarimide and L- and DL-glutamine

(Table from Smith et al, 1965)

<i>Embryotoxic activity of 3-aminoglutarimide and L- and DL-glutamine</i>					
<i>Compound</i>	<i>No. of rabbits</i>	<i>Im-plantations</i>	<i>Re-sorptions</i>	<i>Mal-formed</i>	<i>Normal</i>
3-Amino-glutarimide	4	37	4	0	33
					
DL-Glutamine	5	57	6	0	51
L-Glutamine	5	41	2	0	39
					

Treatment: 150 mg./kg. orally on days 7-12 of pregnancy.

Section 13.1 (carcinogenesis, mutagenesis, impairment of fertility) of the current label for Listed Drug is as follows.

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of L-glutamine.

Studies to evaluate L-glutamine's mutagenic potential have not been conducted.

Animal reproduction studies and its potential for impairment of fertility have not been conducted with L-glutamine. It is also not known whether L-glutamine can

cause fetal harm when administered to a pregnant woman or whether it can affect reproductive capacity.

Given the abundance of L-glutamine in the human body, high levels derived from food products, along with the long clinical history with the Listed Drug, and the proposed SCD patient population, additional nonclinical studies were not needed to support approval of NDA 208587.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

L-glutamine is well characterized chemically, and considered to be a conditionally essential amino acid. Both NDA 208578 and NDA 21667 rely on publicly available scientific literature to describe key aspects of the pharmacology and toxicology of L-glutamine. Of the clinical studies described in the published literature, of note are a study in very-low-birth-weight infants and intensive care unit (ICU) subjects on renal replacement therapy. A range of L-glutamine doses were used in these studies, including the proposed oral L-glutamine dose of 30 g/day. There is a long history of L-glutamine consumption by and administration to humans. In addition, L-glutamine has been studied in a number of clinical conditions and is currently approved for the treatment of SBS. From the perspective of Pharmacology/Toxicology L-glutamine is reasonably safe for use by patients with SCD, and there are no nonclinical approvability issues.

12 Appendix/Attachments

Impurity (b) (4) in NutreStore (L-glutamine powder for oral solution)

Consult: review by Dr. Tamal Chakraborti, PhD (Pharmacologist, DGIEP) (SDN-042; May 26, 2011)

A consult request from the ONDQA dated August 2, 2011, for the revised specification for (b) (4) an impurity in the drug product. The sponsor proposed a new acceptance criterion limit for (b) (4) from (b) (4) % to (b) (4) %.

The following is excerpted from Dr. Chakraborti.

(b) (4)

(b) (4)



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/s/

SHWU LUAN LEE
05/31/2017

CHRISTOPHER M SHETH
06/01/2017