FDA Briefing Document
Oncologic Drugs Advisory Committee Meeting

NDA 208587
L-glutamine
Applicant: Emmaus Medical, Inc.

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We bring the L-glutamine NDA to this Advisory Committee to gain the Committee's insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

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1. PROPOSED INDICATION

The Applicant is seeking regular approval for the following indication:

L-glutamine for the treatment of sickle cell disease

2. EXECUTIVE SUMMARY

On September 7, 2016, Emmaus Medical submitted a New Drug Application (NDA) 208587 for oral L-glutamine powder, seeking approval for the proposed indication of "the treatment of sickle cell disease". The drug is intended for chronic use in adult and pediatric patient's age 5 years and older with sickle cell disease (SCD). The application was granted a standard, 10 month review.

The submission includes the final study report of a phase 3 randomized, multicenter, open-label, double-blind, placebo-controlled study (Study GLUSCC09-01) conducted in patients with SCD or sickle ß$_0$-thalassemia age 5 years and older. In this study subjects with at least 2 episodes of painful crises within the 12 months prior to screening were randomized (2:1) to receive oral L-glutamine 0.3 mg/kg/day or placebo for 48 weeks followed by a 3-week tapering period, and a 2-week follow-up period for a total duration of up to 57 weeks. Randomization was stratified by baseline hydroxyurea (HU) use and geographic region. A total of 230 patients were randomized in the study (152 to L-glutamine; 78 to placebo). One patient randomized to L-glutamine did not receive any study drug. Among the 229 patients treated in the study mean age was 21.9 years, 22.3% of patients were age 12 years or younger, 54.1% were female, 94.3% were Black and 90.4% had diagnosis of sickle cell anemia. Demographic characteristics were comparable in the two treatment arms. Most patients (66.8%) were also receiving HU with a mean of 4.4 years since first treatment, with similar percentages in the two treatment arms. The mean number of crises experienced by patients during the year prior to study entry was 4.0 (median 3.0) with similar numbers in the two treatment arms but a wide range in the number of crises (0 to 18 overall and similar in the two groups).

Efficacy: The primary efficacy analysis compared frequency of sickle cell crises between the two treatment arms during the study. The primary efficacy endpoint was the number of sickle cell crises through Week 48 and prior to start of taper, with a sickle cell crisis defined as a visit to an emergency room/medical facility for SCD-related pain that was treated with a parenterally administered narcotic or parenterally administered Toradol (ketorolac). The protocol-specified primary efficacy population was the intent-to-treat population (ITT) (all patients as randomized). There were additional supporting analyses performed on other defined populations and endpoints.

The statistical analysis of the efficacy results was complicated by a high and differential rate of patient discontinuation from the study before completion of the full 48-week treatment period, which necessitated invocation of imputation methods (as specified in the Statistical Analysis Plan and others) all of which had important limitations due to required assumptions for the

methods. Thus, some caution is warranted in the interpretation of the resulting analyses of the primary efficacy results for Study GLUSCC09-01. FDA's many faceted exploration of a variety of approaches to handling the high and differential dropout rate found that in all the analyses the trend favored L-glutamine over placebo with a range of reduction in crises over 48 weeks from 0.4 to 0.9 crises. The phase 2 study with similar design (Study 10478), which failed to meet its specified significance level for both primary and secondary efficacy analyses, also showed a trend in favor of L-glutamine over placebo.

Safety: The safety population for the indication included 187 patients treated with L-glutamine and 111 patients treated with placebo in the Phase 2 study (10478) and the Phase 3 study (GLUSCC09-01). During the study most patients experienced a treatment-emergent serious adverse event, most commonly sickle cell anemia with crisis (66.3% of patients treated with L-glutamine and 72.1% of patients treated with placebo) and acute chest syndrome (7.0% of patients treated with L-glutamine and 18.9% of patients treated with placebo). Treatment emergent adverse events led to withdrawal from study in 2.7% of L-glutamine treated patients and 0.9% of placebo treated patients. Four deaths occurred in L-glutamine-treated patients and none in placebo-treated patients. One treatment emergent death was due to cardiopulmonary arrest (sudden), one was due to cardiac arrest (no further information provided) and the third was due to respiratory failure, status-post cardiopulmonary arrest, sickle cell crisis, severe anemia and severe hypoglycemia. No autopsies were conducted on any of the mortality cases. No deaths were considered study drug treatment related by the investigators.

ISSUES FOR ODAC

FDA review of this NDA identified the following major issues:

1. Concerns about robustness of efficacy results of Study GLUSCC09-01

Overall, the discontinuation rate in Study GLUSCC09-01 was higher than anticipated (31.9% as compared to expected 25%) and there was a disparate rate of premature discontinuations between treatment arms (36.2% in the L-glutamine arm and 24.4% in the placebo arm). The data and information collected during the study were insufficiently detailed to allow discernment of the reason(s) for the differential higher withdrawal rate in the L-glutamine arm (36.2%). Multiple explorations of ways to handle the missing data yielded findings tending to favor the L-glutamine treatment arm. However, all the methods had significant limitations. The Division of Hematology Products seeks ODAC discussion and perspective on the appropriateness of the statistical methods used in the primary efficacy analysis of Study GLUSCC09-01.

2. Magnitude of any treatment effect of L-glutamine

Estimates of a beneficial treatment effect of L-glutamine over placebo given for 48 weeks in decreasing sickle cell crises ranged from 0.4 to 0.9 crises (mean) or 1 crisis (median [4 to 3]. The

Division of Hematology Products seeks ODAC discussion and perspective on the observed changes.

3. No obvious safety signals

The Division of Hematology Products seeks ODAC comment on adequacy of the safety database for L-glutamine.

The Division of Hematology Products seeks the advice of the ODAC on the question:

Based on the available data presented and discussed, does the ODAC conclude the overall Benefit-Risk profile of L-glutamine for the treatment of sickle cell disease is favorable?

3. BACKGROUND

3.1. Sickle-cell disease

Sickle-cell disease (SCD) is a life-threatening hereditary disorder that affects nearly 100,000 individuals in the United States (Yawn, Buchanan et al. 2014). It is caused by a single point mutation (replacement of glutamic acid with valine) in the in the 6th position of the hemoglobin β-globin chain resulting in the production of mutant hemoglobin molecules (Hemoglobin S [Hb S]). During periods of deoxygenation, Hb S polymerizes within erythrocytes resulting in intermittent vaso-occlusive events and chronic hemolytic anemia. Vaso-occlusion occurs as a result of the formation of multicellular aggregates that block blood flow in small blood vessels, resulting in tissue ischemia & reperfusion damage to downstream tissues which lead to recurrent acute pain episodes (sickle cell crises) and chronic injury affecting any organ system in the body. The most common form of sickle-cell disease (homozygous Hb SS) accounts for 60%-75% of sickle cell disease in the US. Approximately 25% of patients have coinheritance of Hb S with another β-globin chain variant such as sickle-hemoglobin C disease and sickle β-thalassemia. SCD is a multisystem disease associated with profound clinical manifestations. The hallmarks of SCD-related disease are the result of chronic hemolysis and intermittent vaso-occlusive episodes. Vaso-occlusive pain episodes are the most frequent cause of recurrent morbidity in sickle cell disease and account for the majority of sickle cell disease-related hospital admissions (Platt, Thorington et al. 1991, Steinberg 2011). Some patients have few painful events, while others may require hospitalization several times a year. SCD is associated with an overall decreased life expectancy (Platt 1994, Lanzkron, Carroll et al. 2013, Elmariah, Garrett et al. 2014).

Currently, management of SCC episodes is generally supportive and includes symptomatic treatment with analgesics, intravenous fluids, oxygen and RBC transfusion. Hydroxyurea (HU) is the only drug approved to reduce the frequency of SCC in patients with SCD.

3.2. L-glutamine

L-glutamine is conditionally essential amino acid (molecular formula: C5H10N2O3; molecular weight: 146.15) which serves as a building block for proteins in the body. Glutamine is currently approved and marketed under NDA 21,667 as NutreStore® for the treatment of short bowel syndrome (SBS) in patients receiving specialized nutritional support when used in conjunction with a recombinant human growth hormone.

L-Glutamine is the preferred fuel for rapidly dividing cells including hematopoietic cells (Smith 1990), and serves as a precursor of nucleic acids and nucleotides including the pyridine nucleotides, nicotinamide adenine dinucleotide (NAD) and reduced nicotinamide adenine dinucleotide (NADH). These pyridine nucleotides play key roles in the regulation and prevention of oxidative damage in red blood cells (RBCs). Several studies have shown that oxidative phenomena may play a significant role in the pathophysiology of SCD and that sickle RBCs are more susceptible to oxidant damage than normal RBCs. This increased susceptibility to oxidation of sickle RBCs may contribute to chronic hemolysis (Bensinger and Gillette 1974) and vaso-occlusive events in SCD (Hebbel, Boogaerts et al. 1980). In addition, sickle RBCs were

found to have high NAD levels accompanied by a decrease in NAD redox potential, when compared to non-sickle RBCs. This indicated that sickle RBCs may respond to oxidant stress by producing more NAD, but that this response may be overwhelmed resulting in an overall decrease in redox potential (Zerez, Lachant et al. 1988).

In sickle RBCs, there is a higher affinity for and enhanced transport of L-glutamine in, and enhanced conversion of actively transported L-glutamine to glutamate (a byproduct of L-glutamine in NAD synthesis) compared to controls (Niihara, Zerez et al. 1997). The increase of L-glutamine concentration in the intact sickle RBC is thought to further increase the rate of NAD synthetase activity leading to improved NAD redox potential. This concept is supported by the following:

- In vivo analyses demonstrated that glutamine supplementation improved NAD redox potential and resulted in a positive subjective clinical response (Niihara, Zerez et al. 1997).
- Children with sickle cell anemia demonstrate an increase in glutamine utilization of almost 50% when compared to children without sickle cell anemia (Salman, Haymond et al. 1996).
- L-glutamine significantly decreased endothelial cell adhesion in sickle RBCs compared to untreated sickle RBCs in a static human umbilical cord model (Niihara, Matsui et al. 2005).

These data suggest that L-glutamine may provide a clinically protective effect via increased RBC deformability and decreased cell adhesion.

3.3. Key Regulatory History for SCD Clinical Development Program

Key pre-submission and post-submission regulatory issues and interactions between FDA and Emmaus related to the clinical development of L-glutamine for SCD are summarized in Table 3.

Table 1: Key Regulatory Activities

Date	Discussion
01 Aug 2001	L-glutamine granted orphan drug designation by the Office of Orphan
19 Nov 2001	End of Phase 2 meeting held
07 Jan 2005	Granted Fast-Track designation
20 Apr 2009	End of Phase 2 meeting held to discuss the design of the Phase 3 study.
06 Jan 2010	FDA provided advice to the applicant regarding the regarding the clinical and statistical design of the Phase 3 study

Date	Discussion
05 Nov 2012	Type C Meeting held to obtain feedback on the Interim Analysis Report of the Phase 3 study.
11 Jun 2014	Type C Meeting held to obtain the Division's feedback on the proposed NDA plan. Preliminary findings from the Phase 3 trial were presented and discussed.
15 Oct 2014	Type A Meeting held to discuss the analysis of the completed Phase 3 study (Study GLUSCC09-01). FDA expressed concern about …

Source: FDA generated table.

4. CLINICAL STUDIES TO SUPPORT EFFICACY AND SAFETY

This NDA is primarily supported by two randomized, placebo-controlled studies (pivotal phase 3 study GLUSCC0901and supporting phase 2 study 10478) that evaluated the safety and efficacy of L-glutamine for the treatment of SCD in adult and pediatric subjects. The application also included results from 5 other clinical studies (referred to as the legacy studies) conducted by the Applicant in the early stages of the clinical development program of L-glutamine for the sickle cell disease indication. (See Table of Clinical studies below). These additional 5 studies were reviewed as part of FDA's safety evaluation but are not included in the integrated safety database because they were exploratory in nature with small sample sizes, varying study designs and the adverse events were not as explicitly defined as in studies 10478 and GLUSCC0901.

Table 2: Tables of Clinical Studies

Study	Title	Population	Study Design
GLUSCC 09-01	A Phase 3, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study	Patients with Sickle Cell Anemia and Sickle ß0-Thalassemia	Randomized, Double-blind, placebo-controlled, parallel-group
10478	A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Multicenter Study	Patients with Sickle Cell Anemia and Sickle ß0-Thalassemia	Randomized, double-blind, placebo-controlled, parallel-group
8775	Prospective Randomized Crossover Double-Blind Trial	Patients with Sickle cell anemia	Double-blind, placebo-controlled, crossover
8822	A Dose-Finding Study of L-glutamine in Patients With Sickle Cell	Patients with Sickle cell anemia	Dose-finding study
10511	The Effect of L-Glutamine on Exercise Tolerance in Sickle Cell Patients	Patients with sickle cell anemia or sickle β^0-thalassemia	Exercise tolerance study
10779	L-glutamine Therapy Increases Exercise Endurance of Sickle Cell	Subjects with sickle cell anemia or control	Exercise study

8288	Oral L-Glutamine Therapy for Sickle Cell Anemia	Subjects with sickle cell anemia	Pilot study

Source: FDA generated table.

Studies 10478 and GLUSCC0901 share similar features and are described together in section 4.1 below with key differences highlighted.

4.1. Study Design

Studies 10478 and GLUSCC09-01 were both multicenter, open-label, randomized, placebo-controlled studies designed to evaluate the long-term safety and efficacy of L-glutamine for the treatment of SCD in patients with sickle cell anemia and sickle ß^0-thalassemia who were at least 5 years of age.

In both studies, informed consent was obtained up to four weeks prior to Week 0 (Baseline) and screening procedures were performed anytime between the date of consent and Week 0.

Patients were randomized at Week 0 to L-glutamine or placebo. In Study GLUSCC09-01, patients were randomized in a 2:1 ratio (L-glutamine: placebo) and randomization was stratified by investigational site (geographic region) and hydroxyurea usage (yes/no). In Study 10478, patients were randomized in a 1:1 ratio stratified by investigational site but not by HU use. Randomization was double blind with un-blinding only permitted if necessary in case of a medical emergency.

Each 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. In both studies, an equivalent volume of oral powder, L-glutamine or placebo, was administered at a dosage of 0.3 g/kg of subject body weight, twice daily for 48 weeks, with an upper limit of 30 g/day for subjects.

Study medications were self-administered by study patients at home. Study visits occurred monthly. After 48 weeks of treatment, patients were gradually tapered off study medication over a period of 3 weeks before returning for the final visit at Week 53, 2 weeks after the last dose. Throughout the course of the study, clinical and hematological parameters and all adverse events (AEs) were monitored and reported.

The approved anti-sickling agent, hydroxyurea (HU), was permitted in both studies however in GLUSCC0901 randomization was stratified by HU use. All concomitant medications were recorded throughout the course of each study.

4.1.1. Study Population

In Study GLUSCC09-01, 298 patients were enrolled from 31 study sites in the United States. In study 10478, 81 patients were enrolled from 5 study sites in the US.

Except where specified, inclusion and exclusion criteria below are applicable to the patients in both Study 10478 and Study GLUSCC-0901.

Key Inclusion Criteria:
A patient must have met all of the following inclusion criteria to participate in the study

1. Be at least 5 years of age.
2. Be diagnosed with sickle cell anemia or sickle ß°-thalassemia (documented by hemoglobin electrophoresis).
3. Patients should have had at 2 two documented episodes of sickle cell crises within 12 months of the Screening Visit.
4. If the patient was 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.

Key Exclusion Criteria:
Patients who met any of the following criteria were not enrolled:

1. Patients who had a significant medical condition that required hospitalization (other than sickle cell crisis) within two months of the screening visit.
2. Patients had diabetes mellitus with untreated fasting blood sugar > 115 mg/dL (only applicable to Study 10478).
3. Patients who had received any blood products within three weeks of the Screening Visit were ineligible.
4. Patients with uncontrolled liver disease or renal insufficiency were ineligible
5. Pregnant women or lactating patients or patients who had the intention of becoming pregnant during the study were ineligible.
6. Patients currently taking or had been treated with any form of glutamine supplement within 30 days of the Screening Visit.
7. Patients treated with an experimental anti-sickling medication/treatment within 30 days of the Screening Visit (with the exception of hydroxyurea in pediatric patients) were ineligible.

4.1.2 Efficacy and Safety Assessments

For Study GLUSCC09-01:
The *primary efficacy endpoint* was the number of sickle cell crises through Week 48 and prior to start of taper.

A sickle cell crisis was defined as a visit to an emergency room/medical facility for SCD-related pain that was treated with a parenterally administered narcotic or parenterally administered

Toradol (ketorolac). The occurrence of chest syndrome (acute clinical pulmonary findings corroborated by findings of a new pulmonary infiltrate on chest X-ray films), priapism, and splenic sequestration were considered sickle cell crises even if the symptoms were not painful enough to require narcotics or toradol (ketorolac). Splenic sequestration was defined as an increase in spleen size associated with pain in the area of the organ along with a decrease in the hemoglobin concentration of at least 2 g/dL within a 24-hour period.

Secondary efficacy 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 through Week 24 and through 48 (separately)
- Hematological parameters (hemoglobin, hematocrit, and reticulocyte count)

Other efficacy endpoints included height, weight, and growth curve.

Safety Endpoint: The incidence of adverse events (graded according to NCI-CTCAE version 4.03), hematologic and clinical chemistry laboratory parameters, and vital signs.

For Study 10478:

The primary efficacy endpoint was the 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 (from the date/time of registration to the date/time of departure) for an acute sickling-related pain; treated with a parenterally administered narcotic (except for facilities in which only orally administered narcotics were used). 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 (a sudden increase in liver or spleen size associated with pain in the area of the organ, a decrease in the hemoglobin concentration of at least 2 grams per deciliter (g/dL), and, for liver sequestration, abnormal change in liver function tests not due to biliary tract disease) was to be considered a crisis; the occurrence of hematuria and exacerbations of pain was not considered a crisis.

4.1.3. Independent Review

In Study GLUSCC09-01, an independent central adjudication committee (CAC) determined whether reported sickle cell crises events, as well as hospitalizations and emergency room/medical facility visits related to sickle cell crises, met the criteria for efficacy outcomes. The CAC determinations were considered the primary analysis, with the investigator-reported adverse events analyzed secondarily. There was no central Data and Safety Monitoring Board.

In Study 10478, there was no adjudication committee. The primary endpoint was determined at the study site by study investigators.

4.1.4. Statistical Analysis Plan

Study GLUSCC09-01:
Patients were assigned to the treatment groups in a 2:1 (L-glutamine: placebo) ratio. The study was expected to have a 25% dropout rate. The trial was designed to accrue 220 patients (147 patients assigned to L-glutamine therapy and 73 patients assigned to placebo), which would provide 80% power to detect a difference between the groups in the distribution of the number of sickle-cell crises at Week 48 at a significance level of 0.048 using a two-sided test. Power calculations were based on testing of the null hypothesis of no difference in the probability distribution of the number of sickle cell crises at Week 48 between the two treatment groups.

Pre-specified analyses:
For the primary endpoint analysis, a non-parametric analytic method – the Cochran-Mantel-Haenszel (CMH) test (row mean scores) stratified by investigational site and hydroxyurea use, was planned using the rank of the number of sickle cell crises as scores. For patients who discontinue prior to Week 48, sickle cell crisis count was to be imputed using the mean number of crises for the patients of the same treatment group who did complete Week 48. If the imputed count is less than the crisis count at the time of discontinuation, the latter was to be used.

Secondary endpoints included the 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 through Week 24 and through 48 (separately); and hematological parameters (hemoglobin, hematocrit, and reticulocyte count). The safety endpoints were the incidence of adverse events, safety laboratory results, and vital signs.

The same non-parametric method described above for the primary efficacy endpoint analysis was to be used for the analysis of the key secondary endpoints, with the exception of the hematological parameters. For patients who discontinue prior to Week 48, counts were to be imputed in the same manner described for the primary efficacy; for the 24-week time point the imputed count was to be based on the mean number of events for the patients of the same treatment group who did complete Week 24. Pooling of low-enrolling investigational sites was planned prior to unblinding.

One interim analysis was pre-specified and performed when 80 patients had completed 24 weeks of the study. Treatment blind was maintained by having an independent statistician perform the analysis and by using a series of protocol specific procedures (PSPs) to ensure maintenance of blinding. Significance levels for the final analysis accommodated the single interim analysis to preserve the overall type-I error of 0.05.

Final analysis
Significance levels were adjusted to accommodate the single interim analysis and to preserve the overall type-I error of 0.05. A flexible fixed-sequence testing method was used, with the single interim analysis performed at the 0.005 significance level. The significance level for the final depended on the acceptance or rejection of the null hypothesis of the interim analysis. In the final

analysis only the primary endpoint, the number of sickle cell crises at Week 48, was adjusted as described above. All secondary endpoints in the final analysis were supportive of the primary endpoint and were tested at the 0.05 significance level.

Study 10478:
The sample size was based on results from preliminary data and the published 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. The trial was designed to accrue 40 patients per treatment group which would provide 95% power to detect a difference in means of 4.5 (the difference between means of 2.0 for the L-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.

Pre-specified analyses:
The primary efficacy endpoint was the number of sickle cell crises through Week 48 and prior to the start of tapering. The treatment groups were compared with respect to the number of painful sickle cell crises using an analysis of variance (ANOVA) with treatment group and study center in the model.

Secondary efficacy endpoints were – the number of painful sickle cell crises through Week 24, number of hospitalizations 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 (using the RAND 36-Item Health Survey and the Pediatric Quality of Life Questionnaires).

Safety endpoints were the incidence of adverse events, safety laboratory results, and vital signs.

Secondary efficacy analyses included the number of painful sickle cell crises through Week 24 was performed as for the primary efficacy parameter on both the full analysis dataset and the per-protocol dataset. The treatment groups were compared with respect to the number of hospitalizations for sickle cell pain through Week 48 and through Week 24, as well as the number of emergency room visits for sickle cell pain, using an ANOVA with treatment group and study center in the model. This analysis was performed on both the full analysis dataset and the per-protocol dataset. The same approach was used for the number of emergency room visits for sickle cell pain. The other secondary efficacy analyses were performed only on the full analysis dataset.

Safety Analyses: No statistical tests were performed for the safety variables, which included AEs, clinical laboratory evaluations, and vital signs.

Applicant's Changes in the Planned Analyses

- Patients from Site 106 were excluded from the primary analyses due to potential scientific misconduct.

- Methods of analysis were changed for the primary parameter as well as the secondary parameters of number of sickle cell crisis through Week 24, number of hospitalizations for sickle cell pain through Week 24 and through Week 48, and number of emergency room visits for sickle cell pain through Week 24 and through Week 48. The change in methods was made to accommodate the unanticipated number of non-completers and therefore the substantial proportion of imputed data. A nonparametric approach was used, the Cochran-Mantel-Haenszel (CMH), which was planned for a variety of other secondary parameters.
- The planned missing value imputation method for the primary efficacy analysis was replaced by an alternative method. The original methods were developed anticipating a discontinuation rate of no more than 30%; however, approximately 55% of patients in the full analysis dataset did not complete the study. 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. Imputation was documented prior to release of the randomization.
- For analysis of the change from baseline in height, due to the high frequency of "0" values the method of analysis was changed from ANOVA to a Wilcoxon two-sample test using the t-approximation.
- The planned analyses of alcohol usage and tobacco usage were not performed because so few patients used either substance.
- Because of the small number of children enrolled, the growth curve data and the pediatric Quality of Life (QOL) data were provided in listings but the planned analyses were not performed.
- Sensitivity analyses were performed for the primary efficacy analysis by imputing values in a more conservative manner. For this analysis, crises following withdrawal were imputed by using the worst case rate from the subset of completed patients regardless of treatment group. This was performed with and without Site 106, for both the full analysis dataset and the PP dataset.

4.2. Study Results

4.2.1. Disposition of Patients

Disposition of patients for Study GLUSCC09-01 and Study 10478 is summarized in the sponsor's table below:

Table 3: Subject Disposition, All randomized (Study 10478 & GLUSCC09-01)

	Study GLUSCC09-01			Study 10478
	L-Glutamine n=152	Placebo N=78	L-Glutamine N=37	Placebo N=33
Completed Study	97 (63.8)	59 (75.6)	18 (48.6)	12 (36.4)
Discontinued study	54 (36.2)	19 (24.4)	19 (51.4)	21 (63.6)
Reason for discontinuation				
Consent Withdrawn	23 (15.1)	9 (11.5)	3 (8.1)	5 (15.2)
Non compliance	8 (5.3)	1 (1.3)	9 (24.3)	9 (27.3)
Lack of efficacy	-	-	**0 (0.0)**	**1 (3.0)**
Loss to follow-up	5 (3.3)	3 (3.8)	2 (5.4)	1 (3.0)
Adverse events	5 (3.3)	0	0 (0.0)	1 (3.0)
Deaths	2 (1.3)	0	1 (2.7)	0 (0.0)
Initiation of alternative anti-sickling agent	1 (0.7)	0	-	-
Other	11 (7.2)	6 (7.7)	4 (10.8)	4 (12.1)

Source: FDA generated table

4.2.2. Demographic and Baseline Characteristics

Demographic and baseline characteristics for Study GLUSCC09-01 and Study 10478 are summarized in the table below:

Table 4: Demographics, All randomized (Study 10478 & GLUSCC09-01)

Variable	Study GLUSCC09-01		Study 10478	
	L-glutamine (N = 152)	Placebo (N = 78)	L-glutamine (N = 37)	Placebo (N = 33)
Age, in years				
Mean (SD)	22.4 (12.3)	21.4 (12.4)	29.8 (10.7)	27.2 (10.2)
Range	(5 – 57)	(5 – 58)	(11 – 58)	(9 – 55)

Sex [%, (N)]				
Female	52.0 (79)	57.7 (45)	66.7 (22)	34.5 (10)
Male	48.0 (73)	42.3 (33)	33.3 (11)	65.5 (19)
Race [%, (N)]				
Black	94.7 (144)	93.6 (73)	97.0 (32)	96.6 (28)
Hispanic	2.6 (4)	3.8 (3)	3.0 (1)	3.4 (1)
Other	2.6 (4)	2.6 (2)	---	---
Weight, in kg				
Mean (SD)	57.9 (20.3)	55.5 (20.7)	64.5 (14.2)	68.2 (17.2)
Range	(17.5 – 109.1)	(17.7 – 120.9)	(28.3 – 98.0)	(37.6 – 114.3)
Height, in cm				
Mean (SD)	160.2 (18.1)	157.5 (17.0)	167.7 (10.3)	168.4 (10.7)
Range	(106 – 192)	(113 – 187)	(138 – 190.5)	(135 – 193.0)
Hydroxyurea Use [%, (N)]				
Yes	67.1 (102)	66.7 (52)	57.1 (24)	38.5 (15)
No	32.9 (50)	33.3 (26)	42.9 (18)	61.5 (24)
Site/Region [%, (N)]				
101	---	---	28.6 (12)	30.8 (12)
102/103/ 106	---	---	26.2 (11)	28.2 (11)
107	---	---	45.2 (19)	41.0 (16)
Midwest	9.9 (15)	12.8 (10)	---	---
Northeast	31.6 (48)	29.5 (23)	---	---
SA	27.0 (41)	26.9 (21)	---	---
SC	13.8 (21)	14.1 (11)	---	---
West	17.7 (27)	16.7 (13)	---	---

Source: FDA generated table

At study entry in Study GLUSCC09-01 about 67% of patients in both the L-glutamine arm and the placebo arm were on hydroxyurea. In Study 10478 about 62% of patients in the L-glutamine arm were on hydroxyurea as compared to about 39% of patients in the placebo arm among all patients treated (L-glutamine, 37; placebo, 33). In Study GLUSCC09-01 the mean number of sickle cell crises per protocol per patient during prior year at study entry was 3.9 in the L-glutamine arm (median, 3.0; range 0-16) and 4.1 in the placebo arm (median, 3.0; range 0-18). In Study 10478, number of crises during prior year was captured by yes/no answer to the question, 'Has the patient had at least two episodes of painful crises within the last 12 months?'

4.2.3. Efficacy Results

Detailed examination of the efficacy results from the application was conducted by FDA Biometrics and the findings of the statistical review are summarized below. References are listed at the end of this document.

Background

The efficacy of oral L-glutamine therapy for the proposed indication was evaluated in two clinical studies. Phase 2 Study 10478 and Phase 3 Study GLUSCC09-01 were both randomized, double-blind, placebo-controlled, parallel group, multicenter studies that enrolled patients with sickle cell anemia or sickle β^0-thalassemia who are at least five years old and had experienced at least two sickle cell crises within 12 months prior to the screening visit. Data issues in these two studies are considered to be important topics of discussion for the committee. The more supportive study (GLUSCC09-01) had more incomplete data than expected at the time the study was designed. The method used to address the incomplete data in study GLUSCC09-01 appears to be inappropriate and may result in overly optimistic study findings. Discussion of this issue and FDA's analysis follow.

Study 10478 enrolled 81 patients at five sites across the United States. Patients were randomized according to a 1:1 ratio to receive either L-glutamine or placebo therapy for 48 weeks followed by a 3-week tapering period and 2-week follow up period for a total evaluation period of 53 weeks; randomization was stratified by investigational site. The final analysis set excluded 11 patients from one site which the Applicant suspected of study misconduct.

Study GLUSCC09-01 randomized 230 patients according to a 2:1 ratio to either L-glutamine treatment or placebo treatment, respectively, for 48 weeks followed by a 3-week tapering period and a 2-week follow up period; randomization was stratified by investigational site and baseline hydroxyurea use. The Applicant's statistical analysis plan (SAP) specified that the expected study dropout rate was 25% across both treatment groups, and the study was powered at 80% (at a 0.048 significance level) after adjusting for one interim analysis of the number of sickle cell crises at 24 weeks) among completers to detect a difference between treatment groups in the distribution of the number of sickle cell crises at 48 weeks. The number of sickle cell crises for patients who withdrew from the study early was to be estimated by the larger of either the mean number of crises among subjects in the same treatment group who completed the study or the number of crises observed at the time of study discontinuation. The analysis population for Study GLUSCC09-01 is the Intent to Treat (ITT) population, defined as all subjects randomized to a treatment group.

In both studies, the primary efficacy analysis compared sickle cell crisis events after 48 weeks of treatment between treatment arms using the Cochran-Mantel-Haenszel (CMH) test; in Study GLUSCC09-01, the CMH test used modified ridit scores, a non-parametric, rank-based test that compares outcome values weighted by strata size. This test is equivalent to the stratified Wilcoxon rank sum test, first proposed by van Elteren (1960) for stratified or blocked continuous response data, which was used by the Applicant to calculate the study sample size. Secondary

endpoints included the number of hospitalizations and the number of emergency room (ER) visits across the 48-week treatment period. Study GLUSCC09-01 included a 24-week interim analysis of the primary efficacy endpoint as a key secondary endpoint, and the interim analysis did not reach the specified level of significance.

Efficacy results from both studies are summarized in the table below. Study 10478 did not meet its specified significance level for both primary and secondary efficacy analyses. While, the primary efficacy analysis in Study GLUSCC09-01 was statistically significant in favor of L-glutamine treatment, issues with data imputation and analysis overshadow the finding. Additionally, the interim analysis was not significant as a key secondary endpoint; p-values for secondary endpoints listed in the table are considered nominal.

Table 5. Primary and Secondary Efficacy Analysis Results

	Study 10478		**Study GLUSCC09-01**	
Parameter	**L-glutamine N = 33**	**Placebo N = 29**	**L-glutamine N = 152**	**Placebo N = 78**
Primary Efficacy Analysis				
Number of Sickle Cell Crises at 48 weeks				
Mean (SD)	4.5 (5.37)	10.8 (18.74)	3.2 (2.25)	3.9 (2.53)
Median (min, max)	4 (0, 27)	5 (0, 90)	3 (0, 15)	4 (0, 15)
p-value (controlling for study center)	0.076		---	
p-value (controlling for region and HU use)	---		0.005	
Secondary Efficacy Analyses				
Number of Hospitalizations				
Mean (SD)	1.5 (2.46)	2.3 (2.42)	2.3 (1.99)	3.0 (2.31)
Median (min, max)	1 (0, 10)	2 (0, 10)	2 (0, 14)	3 (0, 13)
nominal p-value (controlling for study center)	0.072		---	
nominal p-value (controlling for region and HU use)	---		0.041	
Number of ER Visits				
Mean (SD)	3.7 (5.63)	9.4 (19.91)	1.1 (1.49)	1.6 (2.30)
Median (min, max)	2 (0, 27)	3 (0, 94)	1 (0, 12)	1 (0, 15)
nominal p-value (controlling for study center)	0.129		---	
nominal p-value (controlling for region and HU use)	---		0.128	

SOURCE: Applicant's Integrated Summary of Efficacy, Tables 14, 15, and 16; HU = hydroxyurea use

Although the designs of the two studies were similar, important differences in the definition and classification of the primary endpoint as well as study conduct issues in the Phase 2 study make it difficult to compare results across both studies. Thus, this summary focuses only on the review of the pivotal Phase 3 study, Study GLUSCC09-01. There were three important statistical issues identified during the review of Study GLUSCC09-01 that are summarized below, along with Applicant-submitted and FDA analyses to assess the impact of these issues on study results and interpretation.

Statistical Review Issues

Issue 1: High and differential early study dropout

In both treatment arms of the study, there were considerable and non-ignorable numbers of patients who discontinued the study before the full 48-week treatment period with a higher percentage of patients from the L-glutamine treatment group dropping out of the study early compared to placebo patients. The Applicant's study SAP estimated an overall dropout rate of 25%; in an IND statistical review, the Agency emphasized that a dropout rate higher than 25% as well as differential dropout rates between treatment groups would be a review issue. The Applicant tabulated study completion status and the reasons for early withdrawal from the study in each treatment group; 36.2% of L-glutamine patients dropped out of the study before the full 48-week evaluation period, compared to 24.4% of placebo patients. As summarized in Figure 1 and Table 6, in the L-glutamine treatment group dropout nearly surpassed the expected rate of 25% by Week 24, the midpoint of the 48-week evaluation period. Almost one-third of L-glutamine patients who did not complete the study had withdrawn within 12 weeks of their baseline visit. The timing of dropout between treatment arms is of interest if there is reason to believe that dropout is related to the assigned treatment arm. Listed in Table 7, the two most frequently reported reasons for early withdrawal in both treatment arms were "Consent Withdrawn" and "Other"; these labels and supplemental information provided were not informative for determining the impact of incomplete crisis counts.

Figure 1. FDA Analysis: Study dropout over time by treatment group

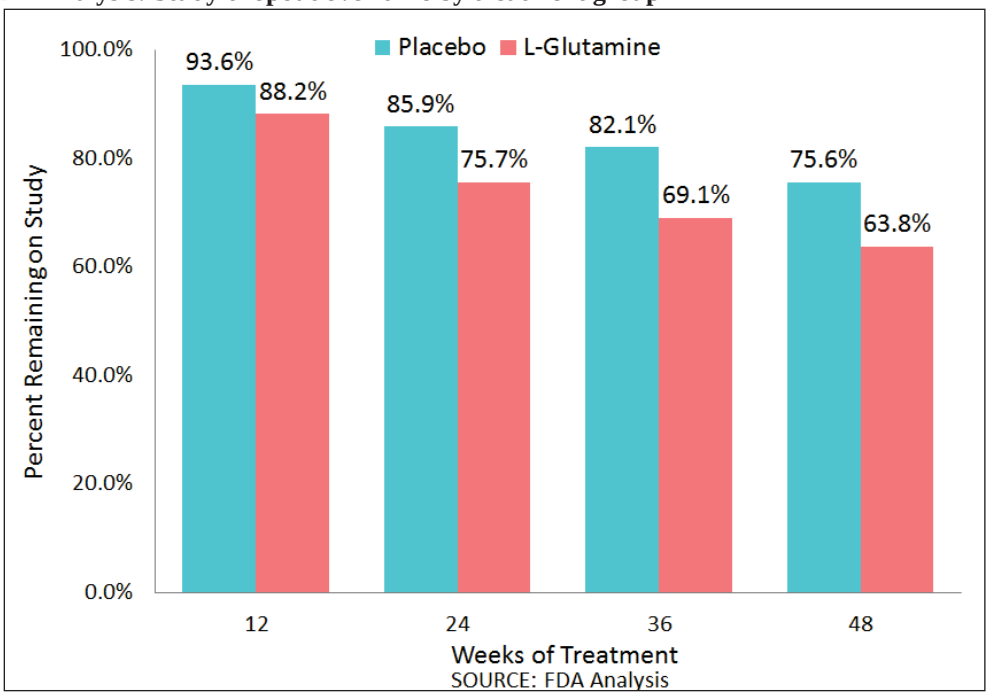


Table 6. Percent of Subjects Remaining on Study GLUSCC09-01, by 12-week intervals and treatment group

		L-glutamine (N = 152)	Placebo (N = 78)
Baseline Visit		100%	100%
Week	12	88.2%	93.6%
	24	75.7%	85.9%
	36	69.1%	82.1%
	48	63.8%	75.6%

SOURCE: FDA Reviewer Analysis

Table 7. Subject Disposition, by Study and Treatment Group, ITT population

	L-glutamine (N = 152)	Placebo (N = 78)
Completed study	97 (63.8)	59 (75.6)
Withdrawn	55 (36.2)	19 (24.4)
Reason for early termination		
Consent withdrawn	23 (15.1)	9 (11.5)
Noncompliance	8 (5.3)	1 (1.3)
Lack of efficacy	---	---
Lost to follow-up	5 (3.3)	3 (3.8)
Adverse events	5 (3.3)	0
Death	2 (1.3)	0
Initiation of alternative anti-sickling agent	1 (0.7)	0
Other	11 (7.2)	6 (7.7)

SOURCE: Applicant's Integrated Summary of Efficacy, Table 13

In an exploratory analysis, FDA summarized dropout patterns by treatment group and study stratification factors in Table 8. In the L-glutamine treatment group, almost half of the patients who were not taking hydroxyurea at baseline withdrew early from the study; by comparison, among placebo patients, dropout rates did not differ as much by baseline hydroxyurea use. Among the five regions where study sites were located, in both treatment groups the highest dropout rates were among patients from South Central US sites followed by South Atlantic site patients.

Table 8: FDA Exploratory Analysis: Percent of early dropouts, by treatment group and study stratification factors

	L-Glutamine N = 152	Placebo N = 78
Overall dropout rate	36.2%	24.4%
By study stratification factors		
Hydroxyurea use		
Yes (n = 153)	29.7%	23.1%
No (n = 77)	49.0%	26.9%
Region		
Northeast (n = 71)	33.3%	21.7%
Midwest (n = 25)	26.7%	20.0%
South Atlantic (n = 62)	39.0%	23.8%
South Central (n = 32)	47.6%	36.4%
West (n = 40)	33.3%	23.1%

SOURCE: FDA Reviewer Analyses

Issue 2: Handling of incomplete sickle cell crisis event counts

In the instance of a high and differential rate of early study dropout between treatment groups, the Applicant's SAP specified that incomplete crisis counts would be imputed as the larger of the mean number of crises for study completers within the same treatment group or the number of crises experienced by the patient at the time of dropout. Considering that nearly one-third of patients had incomplete 48-week counts of sickle cell crisis events, the methods used by the Applicant to impute incomplete counts may have introduced bias in the primary and secondary efficacy results.

The imputation scheme proposed by the Applicant depends on the outcomes of study completers in each treatment group. That is, since the average number of crises experienced by L-glutamine patients who completed the study was three, this number was used as the imputed crisis count for non-completers in the same treatment arm who dropped out with fewer than three crises; in the placebo group, the average number of crises among completers was four, so this number was imputed as the crisis count for non-completers who dropped out with fewer than four crises.

To further examine the impact of how incomplete data were handled, FDA considers four possible patient experiences as represented in the data set of Study GLUSCC09-01and which are listed in the table below; these groups are mutually exclusive and comprise the full ITT study population. For data to be entered into the Applicant's analysis database, a patient had to have experienced a qualifying crisis event. As a result, all "missing" crisis count values were recorded as having a value of zero. Of the 230 patients enrolled in the study, there were 137 patients across both treatment groups who completed 48 weeks of treatment and had at least one crisis event recorded; this is represented in the first row of Table 9. There were 19 other patients who completed the study but did not have any crisis events recorded (Row 2 of Table 9); in this case, it is reasonable to assume that if no crisis event was recorded then no crisis event was experienced. This was explained by the Applicant in response to a request for information from FDA. FDA analyses assume that the final crisis count was zero for patients having such records. For patients who did not complete the study, however, it is not clear from study documentation whether non-completers with no recorded crisis counts have a count equal to zero, missing, or unknown. Thus, we define an FDA sensitivity analysis population consists of the first three rows of Table 9, which represent 206 patients who completed at least 48 weeks of treatment or had at least one crisis recorded at their time of dropout as well as a population that relies on multiple imputation methods to impute counts for 24 patients who did not complete the study and had no crises recorded.

Table 9: FDA Exploratory Analysis: Patient Experiences on Study GLUSCC09-01

	L-Glutamine (N = 152)	**Placebo** (N = 78)
Completed study; at least one crisis recorded	82	55
Completed study; no crises recorded	15	4
Did not complete study; at least one crisis recorded	35	15
Did not complete study; no crises recorded	20	4

Total	152	78

SOURCE: FDA Reviewer Analyses

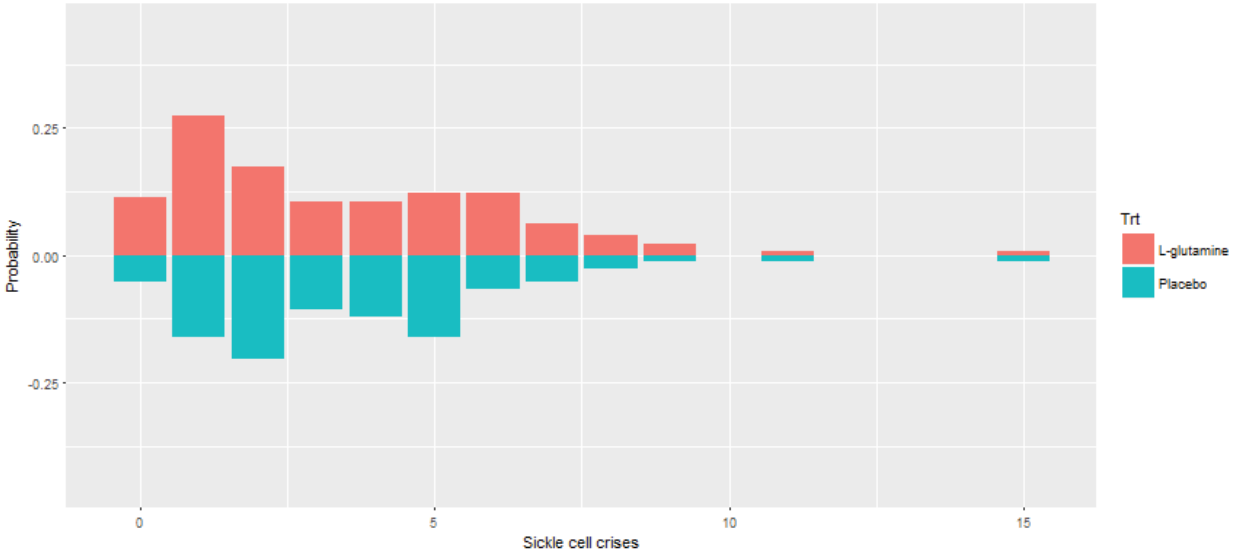
As shown in Table 10, observed (non-imputed) sickle cell crisis counts among all patients ranged from zero to 15 in both treatment groups, and the crisis counts were not normally distributed in either group; using the average crisis count among completers to impute an incomplete crisis count is concerning. The Applicant's imputation method changes the shapes of the distributions of the number of sickle cell crises in each treatment group because of the high number of three's and four's imputed for L-glutamine and placebo non-completers, respectively. Additionally, the imputation method does not take into account other characteristics, such as time spent on the study, or study stratification factors which, when controlled for in the primary efficacy analysis, seem to be associated with the study outcome. The distribution of crises in both treatment groups in the FDA sensitivity analysis population more closely resembles the ITT population with no imputed counts.

Table 10: Exploring the impact of the Applicant's imputation method on the distribution of sickle cell crisis events by treatment group

Number of Crises (Cumulative Percentage)	ITT population		ITT population, Applicant's Imputation Rule		FDA sensitivity analysis population	
	L-glutamine N = 152	Placebo N = 78	L-glutamine N = 152	Placebo N = 78	L-Glutamine N = 132	Placebo N = 74
0	35 (23.0)	8 (10.3)	15 (9.9)	4 (5.1)	15 (11.4)	4 (5.4)
1	36 (46.7)	12 (25.6)	16 (20.4)	10 (17.9)	36 (38.6)	12 (21.6)
2	23 (61.8)	15 (44.9)	17 (31.6)	11 (32.1)	23 (56.1)	15 (41.9)
3	16 (72.4)	8 (55.1)	62 (72.4)	4 (37.2)	16 (68.2)	8 (52.7)
4	16 (82.9)	9 (66.7)	16 (82.9)	23 (66.7)	16 (80.3)	9 (64.9)
5	8 (88.2)	12 (82.1)	8 (88.2)	12 (82.1)	8 (86.4)	12 (81.8)
6	6 (92.1)	5 (88.5)	6 (92.1)	5 (88.5)	6 (90.9)	5 (87.8)
7	5 (95.4)	4 (93.6)	5 (95.4)	4 (93.6)	5 (94.7)	4 (93.2)
8	2 (96.7)	2 (96.2)	2 (96.7)	2 (96.2)	2 (96.2)	2 (95.9)
9	3 (98.7)	1 (97.4)	3 (98.7)	1 (97.4)	3 (98.5)	1 (97.3)
10	0	0	0	0	0	0
11	1 (99.3)	1 (98.7)	1 (99.3)	1 (98.7)	1 (99.2)	1 (98.6)
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

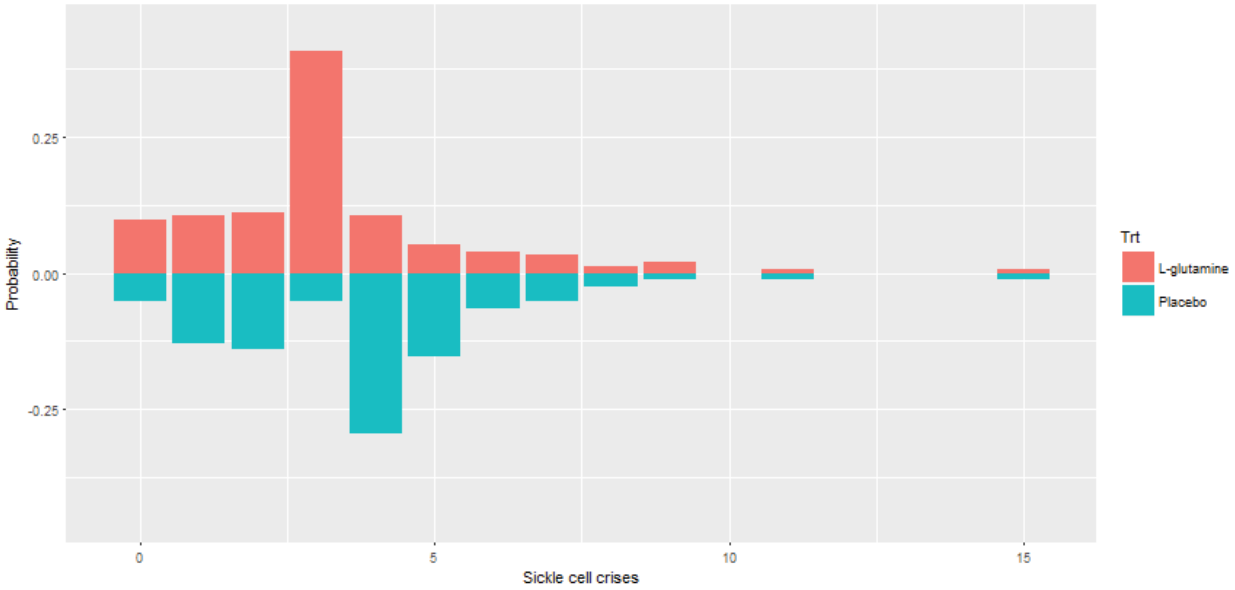
SOURCE: Applicant's Integrated Summary of Efficacy, Tables 1.2 and 4.2; FDA Reviewer Analysis

Figure 2. FDA Analysis: Distribution of sickle cell crisis events by treatment group, FDA sensitivity analysis population, N=206



SOURCE: FDA Reviewer Analysis

Figure 3. Distribution of sickle cell crises by treatment group under Applicant's imputation rule, ITT population, N=230



SOURCE: Applicant's Integrated Summary of Efficacy, Table 1.2

To assess the impact of the originally specified imputation scheme, the Applicant submitted two additional imputation methods: Last observation carried forward (LOCF), and Time-adjusted LOCF. Using the LOCF method, the crisis count for any patient who did not complete the 48-

week treatment period was estimated by their last known crisis count before dropping out of the study. The Applicant's Time-adjusted LOCF method estimates the crisis count at 48 weeks for patients who dropped out early using the number of events at the time of discontinuation divided by the number of days on study medication multiplied by 336. While the Time-adjusted LOCF method attempts to account for time spent on the study, it makes a difficult to justify assumption that the timing between crises takes on a linear trend. In both cases, the mean or median number of crises may not be appropriate measures to describe the data.

Table 11: Applicant Analyses: Number of sickle cell crises (SCC) by methods of imputation

	L-glutamine N=152	**Placebo** N=78
Summary of SCC distribution using Applicant's specified imputation method*		
Mean (SD)	3.2 (2.24)	3.9 (2.54)
Median (min, max)	3 (0, 15)	4 (0, 15)
Alternative imputation method 1: Last observation carried forward (LOCF)		
Mean (SD)	2.5 (2.56)	3.5 (2.74)
Median (min, max)	2 (0, 15)	3 (0, 15)
Alternative imputation method 2: Time-adjusted LOCF		
Mean (SD)	3.6 (4.34)	6.8 (19.09)
Median (min, max)	2 (0, 28)	4 (0, 168)

*Applicant's primary efficacy analysis: CMH test with modified ridit scores, controlling for study stratification factors, nominal p-value=0.0052
SOURCE: Applicant's Integrated Summary of Efficacy, Post Text Tables 4.2 and 5.2

The table above compares each of the alternative imputation methods to the Applicant's original primary efficacy analysis. In each case, both the mean and the median number of crises in the L-glutamine treatment group are lower than in the placebo group.

Issue 3: Interpretation of study results given early study dropout and imputation methods

The third statistical issue of concern is that, given the amount and differential rate of early study dropout as well as the methods used by the Applicant to impute incomplete sickle cell crisis event counts, the analytic method used by the Applicant may not be the appropriate test to demonstrate the benefit of L-glutamine treatment to reduce the occurrence of crisis events among patients with sickle cell disease. The protocol-specified analyses proposed by the Applicant rely on assumptions about the completeness and quality of study data that may not have been met. Since the rate of early dropout may be related to assigned treatment group, time spent on the study, site location, and baseline hydroxyurea use and other unknown factors, interpretation of the primary efficacy analysis is difficult.

Table 12: Applicant Analysis: Results of Applicant's primary efficacy analysis using the Cochran-Mantel-Haenzsel test with modified ridit scoring, ITT population with Applicant's imputation method applied

	L-glutamine N = 152	Placebo N = 78
Number of Sickle Cell Crises at 48 weeks Mean (SD)	3.2 (2.25)	3.9 (2.53)
Median (min, max)	3 (0, 15)	4 (0, 15)
p-value (controlling for region and HU use)	0.005	

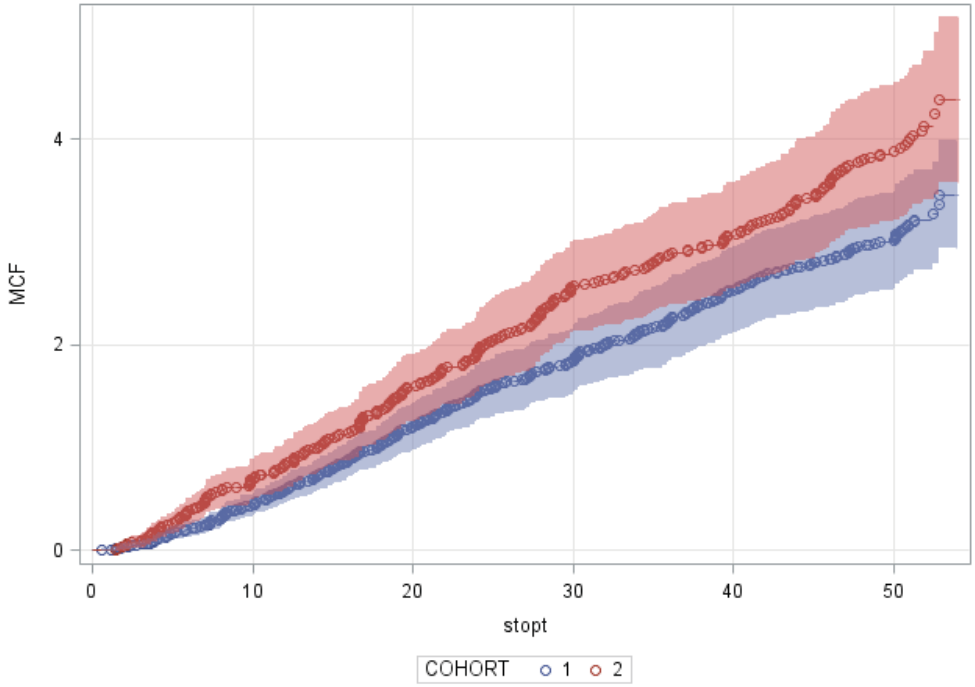
SOURCE: Applicant's Integrated Summary of Efficacy, Table 14; HU = hydroxyurea use

One drawback of the CMH test used by the Applicant is that it ranks the number of crises (according to the Applicant's imputation method) by treatment group and stratification factors without accounting for time patients spent on the study; this complicates interpretation of the primary efficacy results considering that patients from both treatment arms dropped out of the study early, with more L-glutamine patients exiting the study early compared with placebo patients as well as more L-glutamine patients not taking hydroxyurea at baseline withdrawing early compared to other patients.

FDA Data Assessment

An alternative analysis was performed in an effort to overcome the difficulties caused by the incomplete data records. A recurrent event analysis based on the proportional rate regression model (Lawless and Nadeau, 1995; Lin et al., 2000) was performed by FDA to incorporate information on patients' time spent on study and to take into account the fact that times between crisis events for a patient are not necessarily independent. Covariances for the estimators of the regression parameters $\hat{\beta}$, accounting for the dependence structure of the recurrence times, can be computed using a robust (or sandwich) estimator. In this analysis, there is no need for imputation of incomplete crisis counts and all events (as well as timing of events) are included. Patients without any events were censored at their last visit. Based on this analysis, FDA obtained a hazard ratio of 0.73 (95% CI: [0.55, 0.99]) in favor of the L-glutamine treatment group. In the figure below, mean cumulative numbers of crises up to time t (in weeks) is plotted (Nelson, 2003), which is analogous to the Nelson-Aalen estimator for the cumulative hazard function of time to event data. The estimated SCC counts at 48 weeks are 3.8 (95% CI: [3.1, 4.5]) and 3.0 (95% CI: [2.5, 3.4]) for patients in the placebo and L-glutamine and treatment groups, respectively.

Figure 4. FDA Analysis: Mean cumulative functions for sickle cell crisis events by treatment group



Cohort: 1 = L-glutamine; 2 = Placebo; MCF = mean cumulative function, stopt = time (in weeks)
SOURCE: FDA Reviewer Analysis

Table 13: FDA Analysis: Estimated 48-week sickle cell crisis event count by treatment group, recurrent event analysis, ITT population (N = 230)

	L-glutamine N = 152	Placebo N = 78
Estimated sickle cell crisis event count (95% CI)	3.0 (2.5, 3.4)	3.8 (3.1, 4.5)

SOURCE: FDA Reviewer Analyses

The FDA analysis of sickle cell crises as recurrent events takes relevant study information into account to compare the number of crises between treatment groups at Week 48 without requiring imputation of incomplete data, although the analysis requires an assumption of independent censoring. The result favors L-glutamine treatment over placebo.

Sensitivity analyses were performed by FDA using negative binomial regression (NBR), taking into account time spent on study and not requiring imputation of incomplete crisis counts, to compare rates of crises per 48 weeks between treatment groups. First, a NBR analysis was performed using the FDA sensitivity analysis population described previously where incomplete crisis counts for 24 patients (row 4 of table 9 above) were omitted. A second FDA analysis using NBR assumes that incomplete counts for the 24 patients not completing the study were zeros. Finally, a multiple imputation approach, using fully conditional specification (FCS; van Buuren, 2012), imputes counts for the 24 patients excluded from the FDA sensitivity analysis population and applies negative binomial regression as usual. Results of each of the FDA sensitivity

analyses using NBR vary, but together can be interpreted as showing a modest trend supporting the L-glutamine claim of benefit.

Table 14: FDA Exploratory Analyses: Rates of sickle cell crisis counts per 48 weeks between treatment groups, Negative binomial regression

FDA Analysis Set	L-Glutamine N = 132	Placebo N = 74	Rate Ratio for L-glutamine vs. Placebo [95% CI]
FDA sensitivity analysis population (data consisting of rows 1-3 of Table 5 above) (95% CI), N=206	3.3 (2.8, 3.8)	4.1 (3.3, 4.9)	0.80 [0.64, 1.01]
ITT population, assuming crises counts for row 4 in Table 5 are "0" (95% CI), N = 230	3.3 (2.7, 3.9)	4.2 (3.4, 5.1)	0.77 [0.61, 0.99]
Multiple imputation for crises counts in row 4 of Table 5 using fully conditional specification, model also controlling for patient age and baseline crisis count, ITT population (95% CI), N=230	3.9 (3.3, 4.5)	4.3 (3.2, 5.4)	0.91 [0.73, 1.12]

All analyses use time on study as an offset and control for study stratification factors (region of study site and baseline hydroxyurea use)
SOURCE: FDA Reviewer Analyses

Listed in table 16 are two sensitivity analyses considered by the Applicant using negative binomial regression. These analyses also demonstrate a marginal benefit of L-glutamine treatment over placebo, even when taking into account the number of crises experienced by the patient in the year prior to study enrollment.

Table 15: Applicant sensitivity analyses of the primary efficacy endpoint using negative binomial regression, comparing rates of sickle cell crisis events per 48 weeks

Applicant Analysis Set	L-Glutamine N = 152	Placebo N = 78	Rate Ratio for L-glutamine vs. Placebo [95% CI]
ITT population (95% CI), N=230	3.3 (2.7, 3.8)	4.2 (3.4, 5.1)	0.78 [0.61, 0.99]
ITT population, model also adjusting for previous year's crises (95% CI), N=230	3.2 (2.7, 3.7)	4.0 (3.3, 4.9)	0.79 [0.63, 0.99]

Each model includes time on study as an offset, controlling for study stratification factors
SOURCE: Applicant's Response to FDA Statistical Information Request

Overall, when incomplete crisis counts are handled differently than the Applicant's method and analysis of the primary efficacy endpoint takes time on study into account to compare rates of crises between treatment groups after 48 weeks of treatment, the results trend in favor of L-glutamine, but confidence intervals have marginal coverage compared to the planned level of significance.

Summary

In summary, there were more early study dropouts than anticipated in Study GLUSCC09-01, with more dropouts from the L-glutamine treatment group. The amount of incomplete sickle cell crisis event counts in Study GLUSCC09-01 due to high and differential early study withdrawal required imputation of incomplete counts in both treatment groups, and the originally submitted imputation method used by the Applicant appears to be inappropriate. FDA analyses methods that consider an alternative methods of handling incomplete crisis event counts yielded marginal results. In FDA analyses, the reduction in crises over 48 weeks from L-glutamine treatment compared to placebo ranges from 0.4 to 0.9 crises, compared to a difference in 1 crisis resulting from the Applicant's primary efficacy analysis using imputation of incomplete crisis counts. Additional exploratory analyses show a trend in favor of L-glutamine in this setting; this apparent trend should be considered in the context of the safety profile of the product.

4.3. Safety Review

The safety of L-glutamine for the sought indication was based on review of safety data presented in clinical studies GLUSCC09-01 and Study 10478. Details of the protocol design of both studies were described in Section 3. Additional safety information was sought from a systematic review of the published literature.

Safety data from study 10478 and GLUSCC09-01 were included in the pooled integrated safety dataset for analyses. Data from the 5 smaller studies (Studies 8288, 8822, 8775, 10779, and 10511) conducted early in the clinical development program of L-glutamine were not included in the integrated analyses because safety data in these studies were not as explicitly defined or collected as in Study 10478 and Study GLUSCC09-01 (Study 8822 was a dose-finding study and did not collect AE's). See table of clinical trials above.

4.3.1. Safety Population

The safety population consisted of 298 subjects (187 subjects treated with L-glutamine and 111 subjects treated with placebo) who received at least 1 dose of study medication, excluding patients from Site 106 (n=11) in Study 10478. Demographics and baseline characteristics of patients in the safety population are shown in the table below.

The majority of subjects in both treatment groups was black or African American (97.3% in the L-glutamine treatment group and 96.4% in the placebo treatment group) and was 18 years of age or older (57.2% in the L-glutamine group and 56.8% in the placebo group). Ninety percent of subjects in the overall safety population had a diagnosis of sickle cell anemia and were 63.4 percent were being treated with hydroxyurea at baseline (66.3% in the L-glutamine treatment group and 58.6% in the placebo treatment group). In Study 10478, the mean number of SCCs in the year prior to screening was 9.8 and 8.8 in the L-glutamine and placebo treatment groups, respectively. In Study GLUSCC09-01, the mean number of SCCs in the year prior to screening was 3.9 and 4.1 in the L-glutamine and placebo treatment groups, respectively.

Table 16: Demographics, Safety Population (n=298)

	L-glutamine N = 187 n (%)	Placebo N = 111 n (%)	Total N = 298 n (%)
Age (years)			
median	22.0	21.0	22.0
Range (min, max)	(5, 58)	(5, 58)	(5, 58)
Age group, n(%)			
5-12	35 (18.7)	19 (17.1)	54 (18.1)
13-18	45 (24.1)	29 (26.1)	74 (24.8)
>18	107 (57.2)	63 (56.8)	170 (57.0)
Sex, n (%)			
Male	170 (57.0)	53 (47.7)	137 (46.0)
female	103 (55.1)	58 (52.3)	161 (54.0)
Race			
Black	182 (97.3)	107 (96.4)	289 (97.0)
Other[1]	5 (2.7)	4 (3.6)	9 (3.0)
Diagnosis			
Sickle cell anemia	169 (90.4)	99 (89.2)	268 (89.9)
Sickle β0-thalassemia	16 (8.6)	12 (10.8)	28 (9.4)
Other[2]	2 (1.1)	0	2 (0.7)
Hydroxyurea 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.
Source: FDA Reviewer Analysis

4.3.2. Disposition

Overall, 115 subjects (61.5%) in the L-glutamine treatment group and 71 subjects (64.0%) in the placebo treatment group completed the study. Table 17 below shows an overall summary of subject disposition and the reasons for discontinuation among subjects who discontinued the study before the end of follow-up.

Table 17: Disposition of Subjects, Safety Population

Parameter	L-glutamine n = 187 n (%)	Placebo n = 111 n (%)	Total N = 298 N (%)
Completed study	115 (61.5)	71 (64.0)	186 (62.4)
Discontinued study prior to Week 48	72 (38.5)	40 (36.0)	112 (37.6)
Reasons for discontinuation			
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.
Source: FDA Reviewer Analysis

Reasons for discontinuation of treatment were similar between the treatment groups in the safety population. The most common reason for discontinuation in both treatment groups was consent withdrawn (26 subjects [13.9%] in the L-glutamine treatment group and 14 subjects [12.6%] in the placebo treatment group).

FDA reviewed the narratives for patients who withdrew consent. A variety of reasons for withdrawal of consent were reported including – relocation, social issues, "taking too many medication", inability to tolerate powder formulation (bitter), withdrawal of consent by parents/family, fear of interaction with other medications and "feeling worse". FDA also reviewed the verbatim text for subjects treated with L-glutamine who discontinued due to 'other' reasons. Other reasons for study drug discontinuation included pregnancy, relocation to another state, initiating chronic transfusions or bone marrow transplant, initiation of alternative anti-sickling agent and ineligibility for study (detected after enrollment).

4.3.3. Exposure

A total of 298 subjects enrolled in Study 10478 and Study GLUSCC09-01received at least 1 oral dose of L-glutamine or placebo equivalent to 0.3 g/kg administered bid, resulting in daily doses of 30, 20, or 10 g based on subject body weight. The duration of exposure is summarized by treatment group using descriptive statistics in Table 18 below.

Table 18: Summary of Drug Exposure (Safety Population)

	L-glutamine N = 187	Placebo N = 111	Total N = 298
Duration of exposure* (days) Mean (SD)	268.9 (126.92)	283.3 (121.63)	274.3 (124.96)
Subjects with exposure, n (%)			

	L-glutamine N = 187	Placebo N = 111	Total N = 298
≥ 1 day	187 (100.0)		
≥ 12 weeks	161 (86.1)	98 (88.3)	259 (86.9)
≥ 24 weeks	136 (72.7)	89 (80.2)	225 (75.5)
≥ 48 weeks	109 (58.3)	73 (65.8)	182 (61.1
Number of subject-years**	137.7	86.1	223.8

Data Source: Applicants ISS, Table 3
FDA generated table.

Of the 298 subjects included in the safety population, 109 (58.3%) subjects received L-glutamine for ≥ 48 weeks (58.3% in the L-glutamine treatment group and 65.8% in the placebo treatment group). A total of 187 subjects received L-glutamine for ≥ 1 day, 136 subjects received L-glutamine for ≥ 6 months (24 weeks), and 109 subjects received L-glutamine for ≥ 1 year (48 weeks).

4.3.4. Summary of Adverse Events

An overall summary of AEs in all subjects in the safety population is provided in the table below. This is followed by key safety findings regarding deaths, serious adverse events (SAEs), TEAEs that led to study drug withdrawal, common treatment emergent adverse events (TEAEs), TEAEs related to study treatment and clinical laboratory evaluations. TEAEs were 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.

Table 19: Overview of Adverse Events in Safety Population

	L-glutamine N = 187	Placebo N = 111
Subjects with at least 1, n (%)		
TEAEs	180 (96.3)	108 (97.3)
Drug-related TEAEs	35 (18.7)	15 (13.5)
SAE	141 (75.4)	89 (80.2)
Drug-related SAE	3 (1.6)	3 (2.7)
Deaths		0
Total deaths	4 (2.1)	0 (0.0)
Treatment emergent deaths TEAE, n (%)	3 (1.6)	0 (0.0)
Deaths due to drug-related TEAE, n (%)	0 (0.0)	0 (0.0)

Source: FDA Reviewer Analysis

4.3.5. Deaths

Four deaths occurred in safety population. All 4 deaths occurred in L-glutamine-treated subjects. Three deaths were treatment emergent. One death occurred more than 30 days (120 days) after

the last dose of study medication and was not considered treatment emergent. None of the 3 treatment emergent deaths were considered related to L-glutamine treatment by the investigators. The treatment emergent death cases are summarized below and Applicant's narrative for all four deaths are included in the Appendix of this document.

Summary of treatment- emergent deaths

Case 1: Patient 02-504 was a 46 year old African-American female with of sickle cell anemia who was enrolled in Study GlUSCC09-01. She had been treated with oral hydroxyurea 1500mg daily from June 1993 and had two sickle-cell crises in the year prior to enrollment. She and took her first dose of L-Glutamine 15g orally, twice a day) on 13 January 2011.

On (b) (6) she presented to the emergency room "due to acute sickle cell crisis" with Cardiopulmonary resuscitation (CPR) in progress on arrival at the ER. CPR was unsuccessful and the patient was pronounced dead within 30 minutes of arrival to the ER.

While on study, Patient 02-504 was compliant and completed all required study visits for 40 weeks. She had a single episode of vaso-occlusive crisis on September 6, 2011 (week 32) which was treated successfully with concomitant medications and considered un-related to study drug by the investigator. No other significant abnormal findings on physical exams or laboratory results were reported for this patient.

The SAE that led to her demise was reported as sudden death, severe in intensity and not related to study drug. The cause of death was listed as cardiopulmonary arrest. The family declined an autopsy. The Medwatch form did not provided any additional information. FDA requested the death report of this patient from the Applicant however this was not available.

Case 2: Patient 02-516 was a 45-year-old African-American male with sickle-cell anemia who was enrolled in Study GlUSCC09-01 and took his first dose of oral L-Glutamine 10g on January 13, 2011. He participated in the study for 349 days. The last documented day when the patient was still taking study drug was 16 October 2012. He died on (b) (6) from cardiac arrest. No further information was provided. This event was assessed by the investigators as severe in intensity, resulting in fatality, but not related to study drug.

While on study, the following SAEs were reported for this patient:

- Acute infarct/ Transient Ischemia attack (TIA) - moderate in severity, treated with concomitant medications, and resolved with sequelae, not likely related to study drug.
- Acute on chronic renal failure - severe in intensity, treated with concomitant medications, and resolved completely. Not related to study drug.

- Slurred speech
- Abdominal vaso-occlusive crisis (2 episodes).

The information provided is inadequate to determine the cause of death in this patient. The circumstances of his death are unknown. FDA requested the death report of this patient from the Applicant however this was not provided.

Case 3: Patient 101-014 was a 37-year-old woman with sickle cell anemia multiple complications including a history of avascular necrosis of bilateral hips, acute renal failure, aplastic crisis, hemochromatosis, hepatic insufficiency, intermittent seizures, pulmonary hypertension, ankle edema, mild icterus, right toe numbness, systolic murmur, and bilateral edema of the lower extremities. She had been previously treated with hydroxyurea (last dose of HU was in 1994). She had 3 episodes of crises and 3 hospitalizations in the year prior to enrollment. She was enrolled in Study 10478 and took her first dose of L-Glutamine (15g orally, twice a day) on (b) (6). He participated in the study for 331 days. She presented with altered consciousness and hypoglycemia on (b) (6) (Day 331) after 2 days of abdominal pain. She was treated with 50% dextrose and Narcan (naloxone) and was transfused with blood and fresh frozen plasma. She died on (b) (6) after unsuccessful CPR at the ER.

The cause of death listed on her death summary report was 1) Respiratory failure, status-post cardiopulmonary arrest; 2) sickle cell crisis; 3) severe anemia; 4) severe hypoglycemia; 5) liver cirrhosis and 6) renal failure likely secondary to liver failure and hepato-renal syndrome; 7) history of right total hip replacement; 8) history of cholecystectomy and 9) Hypercoagulopathy secondary to liver cirrhosis and End Stage Liver Disease (ESLD). The investigator considered the altered consciousness and hypoglycemia to be severe and unrelated to the study drug. The death report for this patient was requested from the applicant, but this was not available.

Review Comment: The role of L-Glutamine treatment in causing these fatal SAE cannot be categorically ruled out but does not seem likely because: 1) Sickle cell disease is a serious and life threatening disease associated with reduced life expectancy; and 2) Cardiopulmonary complications represent a major mortality risk in adults [Fitzhugh et al 2010].

Summary of non-treatment emergent death

Patient 14-512 was a 10-year-old African-American boy with sickle-cell anemia and a medical history of cholecystectomy, reactive airway disease, delayed hemolytic reaction, iron overload, overweight, icterus, pallor, and a limping gait. He was enrolled in Study GlUSCC09-01 and took the first dose of oral L-Glutamine (10g twice a day) on 29 August 2011. SAEs reported for this patient during his time in study were acute sickle cell pain crises and hematuria. He took his last dose of L-Glutamine on 21 August 2012 and exited from the study on 04 September 2012, having successfully completed follow-up.

On [redacted] (b) (6) he was hospitalized with pain crisis. The following day, his condition worsened and he was transferred to the Pediatric Intensive Care Unit. On [redacted] (b) (6), he went into cardiopulmonary arrest and was pronounced dead at [redacted] (b) (6) on [redacted] (b) (6), death. His cause of death was reported as due to "cardiopulmonary arrest secondary to SIRS, DIC, septic shock, questionable acute abdomen, and HbSS. This SAE was assessed by investigators as not related to study drug.

4.3.6. Nonfatal Serious Adverse Events

The following table provides a summary of adverse events (≥ 2%) in all treated patients who received at least 1 dose of L-Glutamine from all studies included in the integrated safety database. At least one treatment-emergent SAE occurred in 141 subjects (75.4%) in the L-glutamine treatment group and 89 subjects (80.2%) in the placebo treatment group. The most common SAEs occurring the in the L-glutamine group were sickle cell crisis (66.3%), acute chest syndrome (7.0%), and pneumonia (4.8%). Majority of these SAEs were considered unrelated to L-Glutamine treatment by the Applicant. In both the L-glutamine and placebo treatment groups, 3 subjects had at least 1 SAE considered related to study drug by the investigator (1.6% L-glutamine, 2.7% placebo). In the L-glutamine group, SAEs considered by the investigator to be related to study drug included hypersplenism (n=1), sickle cell anemia with crisis (n=1), abdominal pain (n=1), and chest pain (n=1).

Table 20: SAEs occurring in ≥ 2% of L-glutamine-treated Subjects, by PT (Safety Population)

PT	GLN (N = 187)		PLB (N = 111)	
	Number of subjects	Proportion (%)	Number of subjects	Proportion (%)
Sickle cell anemia with crisis	124	66.31	80	72.07
Acute chest syndrome	13	6.95	21	18.92
Pneumonia	9	4.81	10	9.01
Chest pain	5	2.67	2	1.8
Pyrexia	5	2.67	4	3.6
Asthma	4	2.14	3	2.7
Pregnancy	4	2.14	3	2.7

Source: FDA Reviewer analysis

4.3.7. TEAEs that Led to Study Drug Withdrawal

The proportion of TEAEs leading to withdrawal was higher in the L-glutamine treated group (2.7%, 5 subjects) versus the placebo treatment group (0.9%, 1 subject). In the L-glutamine treatment group, 3 subjects (1.6%) had at least 1 TEAE leading to withdrawal that was considered to be related to study drug by the investigator; no subjects in the placebo treatment group had drug-related TEAEs that led to

withdrawal in the L-glutamine treatment group were hypersplenism, abdominal pain, dyspepsia, and hot flush (1 subject each [0.5%]). Of the 5 L-glutamine treated patients who had study drug withdrawn due to a TEAE, 2 patients were using hydroxyurea at baseline.

4.3.8. Common Adverse Events

TEAEs occurring in ≥ 5% of L-glutamine-treated subjects in decreasing order of frequency are shown in the table below. The majority of patients in the L-glutamine treatment group (180 subjects [96.3%]) and in the placebo treatment group (108 subjects [97.3%]) reported at least 1 TEAE. Constipation and nausea occurred more commonly among patients treated with L-Glutamine than among placebo treated patients (constipation 21.4% versus 18.0% and Nausea 19.3% vs 14.4%). Pyrexia and acute chest syndrome occurred more frequently in placebo treated patients. For all other TEAEs, the proportions of subjects reporting events were similar in both treatment groups.

Table 21: TEAEs occurring in ≥ 5% of L-glutamine-treated Subjects, by PT (Safety Population)

	L-glutamine N = *107*		Placebo N = *63*	
PT	Number of subjects	Proportion (%)	Number of subjects	Proportion (%)
Sickle cell anemia with crisis	154	82.35	97	87.39
Constipation	40	21.39	20	18.02
Nausea	36	19.25	16	14.41
Headache	35	18.72	17	15.32
Pyrexia	32	17.11	31	27.93
Cough	29	15.51	15	13.51
Upper respiratory tract infection	26	13.9	20	18.02
Pain in extremity	25	13.37	8	7.21
Vomiting	24	12.83	14	12.61
Back pain	23	12.3	6	5.41
Chest pain	23	12.3	10	9.01
Arthralgia	22	11.76	15	13.51
Abdominal pain	19	10.16	10	9.01
Abdominal pain upper	19	10.16	8	7.21
Acute chest syndrome	19	10.16	24	21.62

Source: FDA Reviewer Analysis

TEAEs in the Legacy studies (Not shown in tables)
In Study 8288, 3 out of 7 patients enrolled experienced at least one AE. The most commonly reported AE was constipation (2 subjects [28.6%]) and hypertension (2 subjects [28.6%]).

In Study 10779, 9 out of 14 patients enrolled (64.3%) experienced at least one TEAE; the most commonly reported TEAEs were sickle cell anemia with crisis (4 subjects [28.6%]) and upper abdominal pain (2 subjects [14.3%]).

In Study 8775, all patients treated with L-glutamine (17 subjects [100%]) and 14/15 patients treated with placebo (93.3) experienced at least one TEAE. The most commonly reported TEAEs were sickle cell anemia with crisis (L-glutamine: 8 subjects [47.1%], placebo: 10 subjects [66.7%]) and constipation (L-glutamine: 8 subjects [47.1%], placebo: 10 subjects [66.7%]).

In Study 10511 4/5 patients treated with L-glutamine (80.0%) and 7/10 patients treated with placebo (70.0%) experienced at least one TEAE. The most commonly reported TEAEs were sickle cell anemia with crisis (L-glutamine: 1 subject [20.0%], placebo: 4 subjects [40.0%]).

4.3.9. TEAEs related to study treatment

Drug related TEAEs occurred in 35 subjects (18.7%) in the L-glutamine group and 15 subjects (13.5%) in the placebo group. Constipation was the most common TEAE considered possibly, probably or definitely related to L-Glutamine treatment. See related TEAEs by treatment group in table below.

Table 22: Related TEAEs, Safety Population

	L-glutamine N = 187		Placebo N = 111	
PT	***Number of subjects***	***Proportion (%)***	***Number of subjects***	***Proportion (%)***
Subjects with ≥1 drug-related TEAE	35	18.7	15	13.5
Constipation	14	7.49	5	4.5
Abdominal pain upper	5	2.67	1	0.9
Nausea	5	2.67	1	0.9
Abdominal pain	4	2.14	4	3.6
diarrhea	3	1.6	1	0.9
Vomiting	3	1.6	3	2.7
Hypersplenism	2	1.07	0	0
Increased appetite	2	1.07	0	0
Pruritus	2	1.07	1	0.9
Sickle cell anemia with crisis	2	1.07	1	0.9

Source: FDA Reviewer Analysis

4.3.10. Laboratory Findings

This section provides a summary of changes in hematological and non –hematological laboratory parameters in the safety population (Study 10478 and GLUSCC0901) as reported by the Applicant and confirmed by FDA analysis.

Hematological parameters

At baseline, mean (SD) hematocrit (HCT) values were similar between the L-glutamine [0.26 (7.5%)] and placebo groups [25 (8.2) %]. RBC count at baseline was also similar between the 2 treatment groups with mean (SD) of 2.80 (0.647) x 10^{12}/L and 2.86 (0.570) x 10^{12}/L, respectively. There was little change from baseline to the end of treatment for HCT and RBC for both treatment groups.

At baseline, mean hemoglobin was slightly lower in the L-glutamine group (131.95 [187.477] g/L) compared with the placebo group (151.82 [220.430] g/L) and the mean (SD) change from baseline to the end of treatment was -13.61 (95.376) g/L in the L-glutamine group and -24.14 (145.731) g/L in the placebo group.

Blood chemistry parameters

Although patients with renal insufficiency were excluded from study 10478 and GLUSCC-0901, majority of patients had some abnormalities in blood chemistry parameters at baseline. Among subjects with low or normal Blood Urea Nitrogen (BUN) values at baseline, 1/132 subjects (0.8%) in the L-glutamine group shifted to a high BUN value at the end of treatment and no subjects in the placebo group shifted to high at the end of treatment. Among patients with low or normal creatinine values at baseline, 4/129 (3.1%) subjects in the L-glutamine group and 1/82 (1.2%) subjects in the placebo group shifted to high at the end of treatment. The table below shows a summary of serum chemistry parameters that shifted to low or high from baseline levels in the Safety population.

Table 23: Summary of Changes in Serum Chemistry Parameters (Safety Population)

	L-glutamine N = 187		Placebo N = 111	
Parameter High or Low	**n (%)**	**Potential to Shift (n)**	**n (%)**	**Potential to Shift (n)**
BUN				
Low	3 (2.5)	118	3 (4.6)	65
High	1 (0.8)	132	0	81
Creatinine				
Low	5 (5.3)	95	5 (8.2)	61
High	4 (3.1)	129	1 (1.2)	82

Studies included: Study 10478 and Study GLUSCC09-01.

The denominators for the percentages are the number of subjects with the potential to shift.

Potential to shift to high = the number of subjects with low or normal values at baseline.

Potential to shift to low = the number of subjects with high or normal values at baseline.

Abbreviations: BUN = blood urea nitrogen.

Source: Copied from Applicants ISS, Table 21.

Potentially Significant Changes in liver function tests (LFTs)
The Applicant's table below shows a summary of outlying values for LFTs (ALT, AST, ALT or AST, total bilirubin, and alkaline phosphatase) for the safety population at the end of study treatment.

Table 24: Summary of Outlying Values for Liver Function Tests (Safety Population

Parameter	L-glutamine N = 187 n (%)	Placebo N = 111 n (%)
ALT		
N with a postbaseline ALT value	132	86
> 3x ULN	2 (1.5)	1 (1.2)
> 5x ULN	1 (0.8)	0
> 10x ULN	0	0
> 20x ULN	0	0
AST		
N with a postbaseline AST value	132	86
> 3x ULN	1 (0.8)	0
> 5x ULN	0	0
> 10x ULN	0	0
> 20x ULN	0	0
ALT or AST		
N with a postbaseline ALT or AST value	132	86
> 3x ULN	3 (2.3)	1 (1.2)
> 5x ULN	1 (0.8)	0
> 10x ULN	0	0
> 20x ULN	0	0
Total bilirubin		
N with a postbaseline total bilirubin value	133	86
> ULN	109 (82.0)	76 (88.4)
> 2x ULN	66 (49.6)	49 (57.0)
Alkaline phosphatase		
N with a postbaseline alkaline phosphatase value	132	86
> 1.5x ULN	4 (3.0)	4 (4.7)

Studies included: Study 10478 and Study GLUSCC09-01.

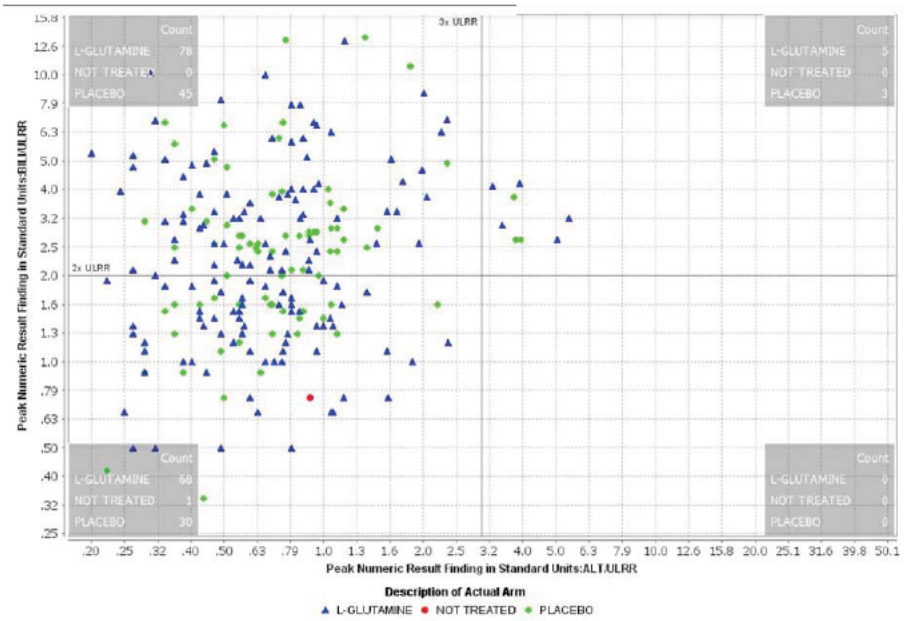
The denominators for the percentages are the number of subjects who had 1 or more postbaseline values for the applicable test(s).

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; ULN = upper limit of normal.

Source: Copied from Applicants ISS, Table 22.

FDA analyzed the laboratory data for subjects in the safety population looking for the occurrence of potential drug-induced liver injury. Five subjects in the L-Glutamine arm and 2 subjects in the placebo arm of Study GLUSCC0901 met the criteria for potential drug-induced liver injury (DILI), as defined by an ALT >3 times ULN, total bilirubin > 2 times Upper limit of normal (ULN), and alkaline phosphatase < 2 times ULN (see Figure 5 below). No potential cases of DLI were identified for subjects enrolled in Study 10478.

Figure 5: Study GLUSCC0901 Potential Hy's Law Plot – Total Bilirubin vs. ALT



Source: FDA Reviewer Analysis

Narratives of subjects in the L-Glutamine arm with potential DILI were reviewed by FDA. All the 5 patients were < 18 years of age (age range: 14-16). All the patients had abnormal liver function tests at base line. All the cases are confounded by multiple medications administered in the course of the study for the treatment of TEAEs. Three patients were receiving HU concurrently.

- One patient (15 year old male) 4-had four episodes of moderate to severe intensity pain crises treated with antibiotics and other supportive medications.
- One patient (15-year-old black female) was diagnosed with viral hepatitis at visit 15.
- One patient (14 year old Hispanic female) had 2 events of severe sickle cell anemia with crisis which resolved with supportive care.
- One patient (16 year old female) was diagnosed with moderate hypersplenism (worsening of anemia and thrombocytopenia) and moderate splenic sequestration.
- One patient (15-year-old, Black female) had 4 episodes of mild to moderate sickle cell anemia with crisis and was hospitalized for multi-organ dysfunction in the course of the study.

4.3.11. Other Safety Explorations

This section provides a summary of TEAE by selected demographic variables (age and gender) for the safety population and by baseline hydroxyurea use.

TEAEs by Age

Both Studies 10478 and GLUSCC09-01 included pediatric patients. FDA evaluated the occurrence of TEAE in the safety population by age (5-12 years, 13-18 years and >18 years). Across both studies, a 54 subjects (18.1%) were 5 to 12 years old, 74 subjects (24.8%) were 13 to 18 years old and 170 (57.0%) were >18 years old. As shown in the following reviewer's table, the percentage of subjects who reported TEAEs was similar in the L-glutamine group and the placebo group for subjects in the 3 age categories.

Table 25: TEAEs by age group, by treatment arm, Safety population

Age group	L-glutamine N=187		Placebo N = 111	
	Number of subjects	Proportion (%)	Number of subjects	Proportion (%)
> 18 years	107	57.22%	63	56.76%
13-18 years	45	24.06%	29	26.13%
5-12 years	35	18.72%	19	17.12%

Source: FDA Reviewer Analysis

The table below shows a summary of TEAEs occurring in ≥ 10% of subjects in the safety population by age (≤ 18 years old and > 18 years old).

Table 26: TEAEs in Subjects ≤ 18 years in the L-glutamine Group, (Safety Population)

TEAEs in Subjects ≤ 18 years				
	L-glutamine N = 187		Placebo N = 111	
PT	Number of subjects	Proportion (%)	Number of subjects	Proportion (%)
Sickle cell anemia with crisis	67	83.75	42	87.5
Constipation	25	31.25	12	25
Pyrexia	22	27.5	23	47.92
Headache	21	26.25	12	25
Cough	20	25	13	27.08
Pain in extremity	18	22.5	4	8.33
Back pain	17	21.25	2	4.17
Abdominal pain	15	18.75	6	12.5
Upper respiratory tract infection	14	17.5	13	27.08
Acute chest syndrome	13	16.25	14	29.17
Nausea	13	16.25	11	22.92
Arthralgia	12	15	8	16.67

Chest pain	12	15	7	14.58
Nasal congestion	11	13.75	4	8.33
Vomiting	11	13.75	8	16.67
Oropharyngeal pain	10	12.5	7	14.58
Ocular icterus	9	11.25	5	10.42
Pruritus	9	11.25	4	8.33
Abdominal pain upper	8	10	3	6.25
Diarrhea	8	10	3	6.25

TEAEs (≥5%) in Subjects ≤ 18 years in the L-glutamine Group, (Safety Population)

	L-glutamine *N = 107*		**Placebo** *N = 63*	
PT	Number of subjects	Proportion (%)	Number of subjects	Proportion (%)
Sickle cell anemia with crisis	87	81.31	55	87.3
Nausea	23	21.5	5	7.94
Constipation	15	14.02	8	12.7
Headache	14	13.08	5	7.94
Vomiting	13	12.15	6	9.52
Pneumonia	13	12.15	12	19.05
Upper respiratory tract infxn	12	11.21	7	11.11
Chest pain	11	10.28	3	4.76
Abdominal pain, upper	11	10.28	5	7.94
Urinary tract infection	10	10	9.35	3
Nasopharyngitis	11	10	9.35	6
Arthralgia	13	10	9.35	8
Pyrexia	13	10	9.35	12
Cough	10	9	8.41	2
Diarrhea	11	9	8.41	4
Leukocytosis	10	9	8.41	5
Fatigue	13	8	7.48	1
Tachycardia	8	8	7.48	3
Pain in extremity	13	7	6.54	4
Anemia	11	7	6.54	9
Dizziness	7	6	5.61	1
Edema peripheral	6	6	5.61	4
Back pain	7	6	5.61	7
Ocular icterus	6	6	5.61	5
Hypokalemia	9	6	5.61	11
Acute chest syndrome	6	6	5.61	16

Source: FDA Reviewer Analysis

The most commonly reported TEAE among subjects who received L-glutamine in all the age group categories was sickle cell anemia with crisis. There were multiple TEAEs where a notable higher percentage of subjects reported that TEAE among subjects ≤ 18 years old compared with subjects > 18 years old in the L-glutamine group: acute chest syndrome (16.3% vs 5.6%), constipation (31.3% vs 14.0%), pyrexia (27.5% vs 9.3%), pain in extremity (22.5% vs 6.5%), back pain (21.3% vs 5.6%), and cough (25.0% vs 8.4%). The percentage of subjects reporting all other TEAEs was generally similar between the age groups in the L-glutamine group.

TEAEs by gender

The percentage of subjects who reported TEAEs was similar for male and female subjects in the L-glutamine group (83 subjects [98.8%] and 97 subjects [94.2%], respectively). The most commonly reported TEAE among for both male and female subjects who received L-glutamine was sickle cell anemia with crisis. Other than nausea which was reported in a higher percentage of female (28 subjects [27.2%]) versus male subjects (8 subjects [9.5%]) in the L-glutamine group; the percentage of subjects reporting all other TEAEs was generally similar between male and female subjects for the L-glutamine group (results not shown).

TEAEs by HU use at baseline

As shown in Table 15, FDA evaluated the occurrence of TEAEs and SAEs in by HU use at baseline in the safety population. Among subjects treated with L-glutamine, the percentage of subjects who reported TEAEs was similar for subjects with HU use at baseline and those without hydroxyurea use at baseline (122 subjects [98.4%] and 58 subjects [92.1%], respectively). However, the percentage of subjects who reported SAEs was slightly higher for subjects with hydroxyurea use at baseline compared with subjects without hydroxyurea use at baseline in the L-glutamine group (100 subjects [80.6%] and 41 subjects [65.1%], respectively). The most commonly reported SAE among all subjects was sickle cell anemia with crisis which was reported in a higher percentage of subjects with hydroxyurea use at baseline compared with subjects without hydroxyurea use at baseline in the L-glutamine group (90 subjects [72.6%] and 34 subjects [54.0%], respectively) and in the placebo group (51 subjects [78.5%] and 29 subjects [63.0%], respectively).The reason for the higher frequency of sickle cell anemia with crisis episodes in HU users in both the L-glutamine and placebo treatment groups is unclear. This could possibly be related to a higher likelihood of seeking medical care or reporting AEs in HU users versus non users in the general population. These data should be interpreted with caution since HU use at baseline was not balanced at baseline in Study 10478.

Among L-glutamine treated patients, overall study discontinuation rates was lower in HU users at baseline (41/124 subjects [33.1%]) than among non- HU users (31/63 subjects [349.2%]) (results not shown in table).

Table 27: AEs by HU use

	L-glutamine N = 187		Placebo N = 111	
Subjects with at least 1, n, (%)	HU use at baseline N=124	No HU use at baseline N=63	HU use at baseline N=65	No HU use at baseline N=46
TEAEs	122 (98.4%)	58 (92.1)		43 (93.5)
TEAEs leading to withdrawal from study	2 (1.6)	3(4.8)	1 (1.5)	0
SAE	100 (80.6)	41 (65.1)	57 (87.7)	32 (69.6)
Sickle cell anemia with crisis	107 (86.3)	45 (71.4)	60 (92.3)	37 (80.4)

Source: FDA Reviewer Analysis

5. MAIN ISSUES WITH THE APPLICATION

Issue 1: Concerns about efficacy findings from Study GLUSCC09-01

Discontinuations and Informative Censoring

A total of 230 patients were randomized to the 2 study treatment groups (2:1 ratio) and received at least one dose of study medication: 151 patients in the L-glutamine group and 78 in the placebo group. Fifty-four patients (35.8%) in the L-glutamine group discontinued the study before the end of follow up (Week 48) compared to 19 (24.4%) in the placebo group. The higher rate of discontinuations in the L-glutamine group is of concern and introduces the possibility of informative censoring. Reasons for discontinuations were however similar between the L-glutamine and placebo groups, with the most frequent being "consent withdrawn" (15.2% [23/151] and 11.5% [9/78] in the L-glutamine and placebo groups, respectively), and "other" (7.3% [11/151] and 7.7% [6/78]).

Missing data and Imputation methods

The rate of discontinuations in GLUSCC0901 was higher than anticipated and resulted in a high proportion of missing data. For patients who discontinued prior to week 48, sickle cell crisis count was imputed using the mean number of crises for the patients of the same treatment group who did complete Week 48, rounded to the nearest integer. If the imputed count was less than the crises count at the time of discontinuation, the latter was used.

Subjects who discontinued the study before the end of follow-up had their number of crises imputed either as the mean for completed subjects of the same treatment group or as the last observation carried forward (i.e., total number of crises at the time of discontinuation), whichever was larger. Since the discontinuation rate was not balanced between treatment arms, the imputation methods used and the impact of the imputation scheme for sickle cell crisis events

in patients who discontinued prior to week 48 is an important biometrics review issue. Sensitivity analyses using different imputation methods were conducted by FDA to determine the effect of imputation methods and statistical considerations on the analysis of the primary endpoint. In general, the sensitivity analysis confirmed statistically significantly fewer SCCs in favor of the L-glutamine treatment group.

Reliability and robustness of the efficacy findings
GLUSCC0901 was designed to detect a difference between the L-glutamine and placebo treatment groups in the distribution of the number of sickle-cell crises at Week 48 at a significance level of 0.048 using a two-sided test based on testing of null hypothesis of no difference in the probability distribution of the number of sickle cell crises at Week 48 between the two treatment groups.

During the Pre-NDA Type C meeting held on June 11 2014, FDA noted that based on the provided results of the phase 3 trial (GLUSCC0901), the pre-specified primary efficacy analysis (using the CMH) test, controlling for region and hydroxyurea use), did not reach the pre-specified significance level of 0.045. In the intent-to-treat 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 (p=0.063). *Furthermore,* the primary efficacy results were inconsistent among geographic regions, as shown by the large difference in results observed based on the stratified analyses adjusted for region and hydroxyurea use (p=0.063) versus results by the analysis adjusted for hydroxyurea use only (p=0.008). In the Type A meeting held between FDA and the Applicant on October 15, 2014, FDA again expressed concern that the findings from GLUSCC0901 were not consistent across the entire study population and the efficacy findings would be more persuasive if supported by an additional confirmatory study.

In the final analysis of the primary efficacy endpoint, presented in this application, a statistically significant improvement in the frequency of SCC in the L-Glutamine treatment arm compared to the placebo arm was demonstrated when a more appropriate CMH model (CMH test with modified ridit scores) was applied and data were stratified by HU use or region, or when no stratification was applied. The median number of SCCs in the L-glutamine treatment group was 25% less or 1 SCC lower than for placebo (p = 0.0052). Sensitivity analyses intended to test the effect of imputation methods also demonstrated statistically significantly fewer SCCs in favor of the L-glutamine treatment group.

Statistically significant fewer hospitalizations for sickle cell pain through Week 48 (median 2 vs 3 in the L-glutamine versus placebo groups; p = 0.041) was also demonstrated for the key secondary efficacy end point; however there was no difference in the number of ER visits for sickle cell pain through week 48 for the treatment groups (median of 1; p = 0.128).

Issue 2: Clinical meaningfulness of observed effect size

In GLUSCC0901, patients receiving L-glutamine experienced a median of 1 fewer SCC compared to those who were not taking L-glutamine within the 48 weeks treatment period. The clinical significance of one fewer sickle cell crises in sickle cell disease patients with a mean number of SCCs in a year of 3.9 is not entirely clear. The FDA is concerned that a decrease of 1 SCC with L-glutamine use observed in the ITT population represents a statistically significant effect that may not be considered clinically meaningful

However, this finding is supported by results of the secondary efficacy endpoint analyses. The observed median number of hospitalizations for sickle cell pain of approximately 33% lower or 1 fewer hospitalization for sickle cell pain in the L-glutamine compared to the placebo treatment group ($p = 0.041$). The mean number of ACS occurrences was approximately 67% or 0.2 fewer for the L-glutamine group than for placebo treatment group ($p = 0.0028$). L-glutamine also delayed the onset of the first SCC. In Study GLUSCC09-01, the crisis-free survival probability was greater in the L-glutamine treatment group throughout the duration of the study relative to the placebo group ($p = 0.0152$), and the point estimates for the 25th, 50th, and 75th quartiles of the crisis -free survival curve were higher in the L-glutamine treatment group.

Issue 3: Safety

There were 3 treatment-emergent deaths associated with L-glutamine use. None of the deaths were considered by the investigator to be related to study drug. FDA reviewed narratives of the cases and available death reports of these cases. These cases were confounded by the multiple co-morbidities and concomitant medications taken by the subjects. In the absence of autopsy findings, FDA is unable to comment on the relatedness of these events to L-glutamine treatment. No deaths were reported in the placebo group. Other than sickle cell anemia with crisis which occurred in majority of L-glutamine and placebo treated patients, the most commonly reported TEAEs were constipation (21.4% L-glutamine and 18.0% placebo), nausea (19.3% L-glutamine and 14.4% placebo), headache (18.2% L-glutamine and 15.3% placebo), pyrexia (17.1% L-glutamine and 27.9% placebo), and cough (15.5% L-glutamine and 13.5% placebo). TEAEs were considered to be related to study drug in 18.7% of subjects in the L-glutamine treatment group 13.5% of subjects in the placebo treatment group by the investigator. Treatment-emergent AEs leading to study drug discontinuation occurred in 5 subjects (2.7%) in the L-glutamine treatment group and 1 subject (0.9%) in the placebo treatment group.

Overall, there were few notable differences in the percentages of subjects who reported TEAEs, SAEs, or TEAEs that led to withdrawal between the L-glutamine and placebo groups for subgroups of subjects evaluated by sex, age, race, diagnosis at baseline, or hydroxyurea use at baseline. No notable changes in clinical chemistry or hematology parameters were observed.

6 Issues for the Advisory Committee

Issues for Advisory Committee consideration for this application include:

1. **Concerns about robustness of efficacy results of Study GLUSCC09-01**

   Overall, the discontinuation rate in Study GLUSCC09-01 was higher than anticipated (31.9% as compared to expected 25%) and there was a disparate rate of premature discontinuations between treatment arms (36.2% in the L-glutamine arm and 24.4% in the placebo arm). The data and information collected during the study were insufficiently detailed to allow discernment of the reason(s) for the differential higher withdrawal rate in the L-glutamine arm (36.2%). Multiple explorations of ways to handle the missing data yielded findings tending to favor the L-glutamine treatment arm. However, all the methods had significant limitations. The Division of Hematology Products seeks ODAC discussion and perspective on the appropriateness of the statistical methods used in the primary efficacy analysis of Study GLUSCC09-01.

2. **Magnitude of any treatment effect of L-glutamine**

   Estimates of a beneficial treatment effect of L-glutamine over placebo given for 48 weeks in decreasing sickle cell crises ranged from 0.4 to 0.9 crises (mean) or 1 crisis (median [4 to 3]. The Division of Hematology Products seeks ODAC discussion and perspective on the importance of these changes.

3. **No obvious safety signals**

   The Division of Hematology Products seeks ODAC comment on adequacy of the safety database for L-glutamine.

The Division of Hematology Products seeks the advice of the ODAC on the question:

Based on the available data presented and discussed, does the ODAC conclude the overall Benefit-Risk profile of L-glutamine for the treatment of sickle cell disease is favorable?

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