ORAL L-GLUTAMINE POWDER FOR THE TREATMENT OF SICKLE CELL DISEASE

NDA 208587

SPONSOR BRIEFING DOCUMENT

ONCOLOGIC DRUGS ADVISORY COMMITTEE

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ADVISORY COMMITTEE BRIEFING MATERIALS

AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACS	acute chest syndrome
AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
BID	twice a day
bpm	beats per minute
BUN	blood urea nitrogen
CAC	central adjudication committee
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CMH	Cochran-Mantel-Haenszel
CSR	clinical study report
ER	emergency room
FDA	Food and Drug Administration
H_0	null hypothesis
HBB	hemoglobin beta
HbS	abnormal hemoglobin
HCUP	Healthcare Cost and Utilization Project
Hgb SC	sickle cell trait
HR	hazard ratio
HU	hydroxyurea
IND	Investigational New Drug
ISE	Integrated Summary of Efficacy
ITT	intent-to-treat
Km	Michaelis-Menten constant
L-gln	L-glutamine
LOCF	last observation carried forward
LS	least squares
MedDRA	Medical Dictionary for Regulatory Activities
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NBR	negative binomial regression
NDA	New Drug Application
NIH	National Institutes of Health
Pbo	placebo
PK	pharmacokinetics
PT	preferred term

RBC	red blood cell
SAE	serious adverse event
SBS	short bowel syndrome
SCC	sickle cell crisis
SCD	sickle cell disease
SD	standard deviation
SE	standard error
SOC	system organ class
TEAE	treatment-emergent adverse event
tid	three times a day
ULN	upper limit of normal
US	United States

1 EXECUTIVE SUMMARY

The Oncologic Drugs Advisory Committee is being convened to discuss new drug application (NDA) 208587 for orally administered L-glutamine powder. The proposed indication is treatment of sickle cell disease (SCD), in adults and children at least 5 years of age.

L-glutamine is an amino acid formulated as a white crystalline powder and is packaged in 5 grams packets. The proposed dose is 0.3 gram/kg (10, 20, or 30 grams daily based on weight) in both adults and pediatric patients. L-glutamine is currently approved and marketed under NDA 21,667 as NutreStore® (L-glutamine powder for oral solution) for the treatment of short bowel syndrome (SBS) when used in conjunction with a recombinant human growth hormone.

Aside from being a building block for protein, glutamine is a precursor for nicotinamide adenine dinucleotide (NAD) and is essential for the formation of the antioxidant reduced nicotinamide adenine dinucleotide (NADH) (Smith and Wilmore, 1990b). Nicotinamide adenine dinucleotide redox potential (defined by the ratio of NADH to total NAD) is significantly lower in sickle red blood cells (RBCs) (Zerez et al, 1988). L-glutamine-induced increases in redox potential may reduce oxidative stress in these cells, resulting in decreased RBC adhesion, decreased vaso-occlusion, and thus, fewer painful sickle cell crises (a debilitating outcome of SCD). Fewer sickle cell crises translate into decreased mortality in SCD.

1.1 Sickle Cell Disease is a Rare and Serious Disease

Sickle cell disease is a rare hereditary disease caused by a point mutation in the hemoglobin beta (HBB) gene resulting in the formation of hemoglobin S.12. The change alters the physical properties of deoxygenated hemoglobin, causing polymerization of the molecule within RBCs. These hemoglobin polymers lead to increased rigidity and damage to the cell membrane with resultant increased adherence to endothelial membranes. These changes trigger vaso-occlusion. The vaso-occlusion of SCD is associated with profound clinical manifestations the most common of which are acute ischemic painful episodes (> 90% of patients) and acute chest syndrome (ACS) (> 50%) (Steinberg et al, 2011). Other common manifestations include stroke, crippling and painful osteonecrosis, proliferative retinopathy, splenic infarction, leg ulcers, infection, and psychosocial issues.

Approximately 100,000 Americans in the United States (US) suffer from SCD (Centers for Disease Control and Prevention [CDC], 2017). They are mostly of African descent and more rarely of Middle Eastern and Hispanic descent. Treatment of SCD is financially burdensome for patients and payers. In 2006, in the US, there were an estimated 230,000 emergency room (ER) visits and combined emergency room and hospital inpatient charges for SCD patients were estimated to have cost $2.4 billion (Lanzkron et al, 2010). In a retrospective a survey of 21,112 SCD patients, almost 110,000 hospital stays or ER visits per year were recorded for an average of 2.59 encounters per patient year (Brousseau et al, 2010). Hospital stays for SCD are largely billed to public payers with 66% paid by Medicaid and 13% paid by Medicare between 1994 through 2004 (Steiner, 2006).

The majority of ER visits and hospitalizations in the SCD population result from sickle cell crisis (SCCs). Sickle cell crises are intermittent episodes of vaso-occlusion in connective and musculoskeletal structures resulting in severe painful ischemia manifested by acute pain and tenderness, fever, tachycardia, and anxiety (Kasper et al, 2005). Management of SCCs typically includes hydration, aggressive pain management with both long- and short-acting opioids, and transfusions. While frequency of SCCs varies among patients, each crisis is considered a significant event due to the severity of the pain, impact on quality of life, and associated decrease in overall survival (Platt et al, 1991; Hillman et al, 2011).

Importantly, the overall survival of patients in the U.S. with sickle cell anemia is correlated with disease state severity as measured by number of SCCs experienced by patients annually. Patients who experience more than 3 crises per year are more likely to experience fatal complications during their 30's and 40's in comparison to a median survival of nearly 50 years in patients who experience between 1 and 3 crises per year (Platt et al, 1994). Among Black Americans with SCD, the disease causes a decrease in life expectancy of 25 to 30 years in comparison to the Black American population in general (Platt et al, 1994).

1.2 Unmet Medical Need

Sickle cell crisis is a serious and potentially life-threatening consequence of SCD and lowering the frequency of these events remains a major unmet need in SCD patients. Hydroxyurea (HU) is the only drug approved by the Food and Drug Administration (FDA) to reduce the frequency of SCC. Hydroxyurea was first approved for treatment of malignancies in 1967, and the SCD indication was added in 1998 for adult use (Droxia® USPI, 2016). There are currently no approved therapies for reduction of SCCs in children with SCD in the US. This is an area of critical need because the symptoms and organ damage of SCD begin early in life, including stroke in 10% of pediatric patients (Verduzco and Nathan 2009). Silent strokes leading to brain damage and cognitive impairment are also prevalent. There is an urgent need for an agent that can modify the course of disease for SCD children.

In adult SCD patients, treatment with HU is approved for reduction in the frequency of SCC. HU reduces, but does not eliminate SCCs. Many patients treated with HU will continue to experience SCCs at a frequency that places them at considerable risk for organ damage and early death. In addition, some patients cannot tolerate treatment with HU due to hematologic complications and other reasons (Droxia® USPI, 2016). Thus, in adult SCD patients there is an unmet need both for treatments that can be taken in combination with HU, and for treatment in patients who cannot tolerate HU.

There is a clear need for additional therapies to effectively and safely reduce the incidence of SCCs in both adult and pediatric populations.

1.2.1 Clinical Development of L-Glutamine

L-glutamine is one of the most ubiquitous amino acids, with a high utilization rate (Wernerman and Hammarqvist, 1994). Aside from being a building block for protein, glutamine also serves as a precursor of nucleic acids and nucleotides including the pyridine nucleotides, NAD and NADH, that play key roles in the regulation and prevention of oxidative damage in RBCs (Smith and Wilmore, 1990b, Jaffe, 1974).

Several studies have shown that oxidative phenomena plays a significant role in the pathophysiology of SCD leading to oxidant damage (Asakura et al, 1977; Campwala and Desforges, 1982; Chiu et al, 1979; Das and Nair, 1980; Hebbel et al, 1982; Jain and Shohet, 1984). This increased oxidant stress in sickle RBCs may contribute to chronic hemolysis (Bensinger and Gillette, 1974) and vaso-occlusive events in SCD (Hebbel et al, 1982). These changes were reflected with decreased NAD redox potential (Zerez et al, 1988).

The biological plausibility of L-glutamine in the treatment of SCD is further summarized by the following:

- In vivo analyses demonstrated that glutamine supplementation improved NAD redox potential and resulted in a positive subjective clinical response (Niihara 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 et al, 1996).
- L-glutamine at a dose of 30 grams daily for at least 4 wks significantly decreased endothelial cell adhesion in sickle RBCs compared to untreated sickle RBCs in a static human umbilical cord model (Niihara et al, 2005). In all patients treated with L-glutamine, there was large decrease in adhesion rate ($p < 0.001$).

Collectively, these data suggested that L-glutamine may provide a clinically protective effect via increased RBC integrity and decreased cell adhesion

Based on the biological plausibility established in these studies, investigator-initiated trials evaluating L-glutamine for the treatment of SCD began with support from the National Institutes of Health (NIH) and the FDA Orphan Drug Office. These legacy studies are summarized in Section 4. Importantly, the first two legacy studies conducted in SCD patients, Study 8288 and Study 8822, demonstrated significant changes in both the NADH level and NAD redox potential at a daily dose of 30 grams, suggesting that the 30 gram/day dose would be optimal for clinical trials in patients with SCD.

Based on the promising results of the legacy studies, Phase 2 Study 10478 and Pivotal efficacy Study 09-01 were conducted to evaluate the efficacy and safety of L-glutamine in SCD patients (Section 5). Along with these clinical milestones, L-glutamine was granted an orphan drug designation for the treatment of SCD in 2001, and a Fast Track designation in 2005. Throughout clinical development, Emmaus has worked closely with the FDA to establish supportive non-clinical pharmacology and toxicology data, confirm the clinical pharmacology knowledge, confirm the suitability of the design and dosing in the Phase 2 and Phase 3 studies, and discuss

the statistical analysis of the Phase 3 study. Details of these regulatory interactions are summarized in Section 4.

1.3 Efficacy of L-Glutamine for the Treatment of SCD

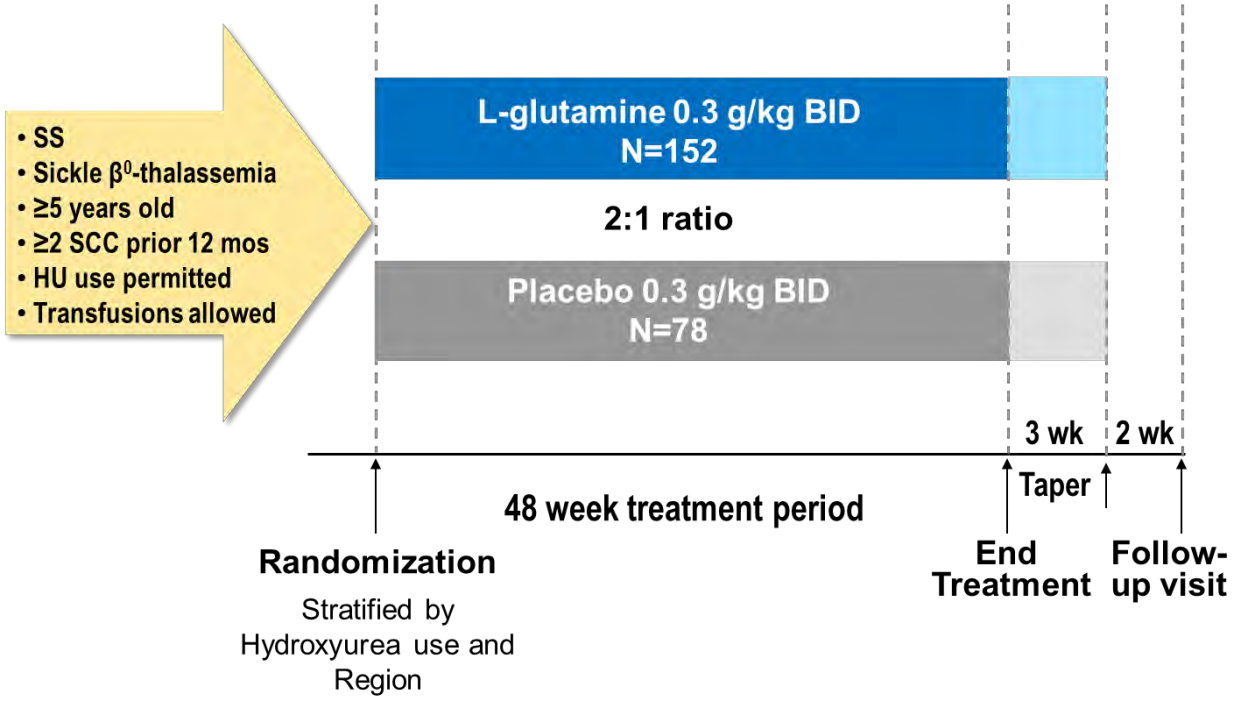
Based on the promising results observed in the legacy studies, the non-pivotal prospective randomized, double blind, placebo-controlled Phase 2 Study 10478 was conducted. With the positive trends from Study 10478, an appropriately powered Phase 3 Study (09-01) was undertaken. A comprehensive review of the pivotal Phase 3 Study 09-01 is presented below followed by an abbreviated summary of Study 10478 results, and concluding with the overall efficacy findings.

1.3.1 Study Design

The pivotal efficacy study was a randomized, double-blind, placebo-controlled, parallel-group, multicenter study evaluating the long-term safety and efficacy of oral L-glutamine therapy for adult and pediatric patients who were at least 5 years of age, with sickle cell anemia or sickle β^0-thalassemia (Figure 1). Patients were required to have had at least 2 episodes of painful crises, with no upper limit for eligibility, within the 12 months prior to the screening visit. Concomitant HU use was permitted in the study as were blood transfusions.

This study consisted of a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period. Patients were randomized in a 2:1 ratio (L-glutamine versus placebo) and stratified by HU usage and by site (region). Approximately 0.3 gram/kg of L-glutamine or placebo (100% maltodextrin) of equivalent volume was administered orally twice daily for 48 weeks. The dosage was in increments of 5 grams and the total daily dose was 10, 20, or 30 grams based on weight (Section 5, Table 11). Study visits occurred every 4 weeks and compliance was monitored in between visits via phone calls and patient diaries. Inclusion/exclusion criteria are shown in Section 5.1.1.

Figure 1. Study Design - Study 09-01



BID = twice a day, HU = hydroxyurea, SCC = sickle cell crisis, SS = sickle cell disease

The primary objective in 09-01 was to demonstrate a difference in the number of SCCs between the randomized groups (Section 5.1.3). The primary efficacy endpoint was the number of sickle cell crises. A SCC was defined as a visit to an emergency department or medical facility for SCD-related pain that was treated with a parenterally-administered narcotic or parenterally-administered ketorolac (Table 14). In addition, the occurrence of ACS, priapism, and splenic sequestration were considered sickle cell crises (Section 5.1.4.3).

Sickle cell crises were recorded on the adverse event (AE) case report forms. An independent central adjudication committee (CAC) evaluated whether reported adverse events of SCCs met the criteria of the efficacy outcome. The committee was composed of three physician members (hematologists and oncologists) that followed procedures detailed in a CAC Manual.

Additional efficacy endpoints specified in the integrated summary of efficacy (ISE) included time to first crisis, occurrences of ACS, number of hospitalizations for sickle cell pain, percentage of time hospitalized, number of ER visits for sickle cell pain, and hematologic parameters (Section 5.1.3). Time to second crisis and frequency of blood transfusions were evaluated as additional *post hoc* analyses.

The primary efficacy analysis population was the intent-to-treat (ITT). The Wilcoxon rank-sum test was used to determine a sample size of 220, with 147 patients assigned to L-glutamine therapy and 73 patients assigned to placebo (Section 5.1.4.1). For the primary statistical analysis

method, the Cochran-Mantel–Haenszel (CMH) test with modified ridit application was chosen. The test of the null hypothesis of the primary endpoint in the final analysis was a two-sided comparison at an overall alpha level of 0.045 (reduced from 0.050 due to an interim analysis). Details on sensitivity analyses are provided in Section 5.1.4.3.

1.3.2 Results

A total of 230 patients were randomized. Of these, 229 received at least 1 dose of study medication and were summarized for safety. A total of 152 patients were randomized to the L-glutamine group and 78 to the placebo group. Of these, 156 patients completed the study, 63.8% (97/152) of those in the L-glutamine group and 75.6% (59/78) of those in the placebo group. While there was a more than 10 point difference in the percent of patients terminating the trial early (36.2% in the L-glutamine group and 24.4% in the placebo group), sensitivity analyses demonstrated that the primary results of the trial are robust against this difference in early termination (Section 5.2.3). Reasons for discontinuation were similar between the groups, with the most frequent being consent withdrawn (15.1% [23/152] and 11.5% [9/78] in the L-glutamine and placebo groups, respectively), and "other" (7.2% [11/152] and 7.7% [6/78] in the L-glutamine and placebo groups, respectively). Items in the categories "consent withdrawn" and "other" included many logistical reasons, ie patient relocation and bone marrow transplant (Section 5.2.1).

Age and distribution by race and by diagnosis were similar between the treatment groups (Table 1). Notably, a large percentage of pediatric patients were enrolled into this study with 49% (75/152) of the patients in the L-glutamine group and 55% (43/78) of the patients in the placebo group being 18 years old or younger. The gender distribution was majority female in both groups.

Table 1. Demographic and Baseline Characteristics – Study 09-01

	L-glutamine N = 152	**Placebo N = 78**
Age (years)		
Mean (SD)	22.4 (12.32)	21.4 (12.42)
Range	5 - 57	5 - 58
Groups, n (%)		
≤ 18 years	75 (49.3)	43 (55.1)
> 18 years	77 (50.7)	35 (44.9)
Sex, n (%)		
Male	73 (48.0)	33 (42.3)
Female	79 (52.0)	45 (57.7)
Race, n (%)		
Black	144 (94.7)	73 (93.6)
Hispanic	4 (2.6)	3 (3.8)
Caucasian	--	--
Asian	--	--
Other	4 (2.6)	2 (2.6)
SCCs in the year prior to screening, n	152	78
Mean (SD)	3.9 (2.7)	4.1 (2.8)
Prior treatment with HU		
Yes, n (%)	101 (66.4)	52 (66.7)

HU = hydroxyurea, SD = standard deviation, SCC = sickle cell crisis

Compliance was measured by the number of days on study and percentage of study medication taken. The median number of days on study was similar in the placebo group and the L-glutamine group. The percentage of study medication taken was the same in the 2 groups at about 75%.

Efficacy Analysis

The results of the primary analysis were statistically significant and demonstrated fewer SCCs in favor of the L-glutamine treatment group relative to placebo (P =0.0052).

The mean and median numbers of SCCs through Week 48 are presented in Table 2. The mean number of crises was 3.2 in the L-glutamine group vs 3.9 in the placebo group. The median number of SCCs in the L-glutamine treatment group was 25% less or 1 SCC lower than for placebo. Sensitivity analyses demonstrated that these results were consistent when controlling only for HU use, only for region, or neither. Additionally, a negative binomial regression (NBR) analysis was conducted on the primary endpoint to confirm the consistency of the result when imputation was not applied. The NBR method also allowed for an estimation of the treatment effect. This analysis also demonstrated a statistically significant lower rate of SCCs in the L-glutamine treatment group relative to the placebo group (p = 0.0374) with a rate ratio (0.78) in favor of patients that received L-glutamine.

Table 2. CMH and NBR Analyses of the Number of SCCs - Study 09-01

	L-glutamine N = 152	Placebo N = 78
Primary Endpoint: Number of SCCs using modified ridit scores and CSR imputation rules		
Primary analysis[a]:		
P-value (controlling for region and HU use)	0.0052	
Descriptive statistics		
Mean (SD)	3.2 (2.24)	3.9 (2.54)
Median (min, max)	3.0 (0, 15)	4.0 (0, 15)
NBR Modeling[b] Results		
P-value	0.0374	
Rate per 48 weeks (95% CI)	3.25 (2.76, 3.83)	4.19 (3.44, 5.11)
Rate ratio[c] (95% CI)	0.78 (0.61, 0.99)	

CI = confidence interval, CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, HU = hydroxyurea, ITT = intent-to treat, NBR = negative binomial regression, SCCs = sickle cell crises, SD = standard deviation.

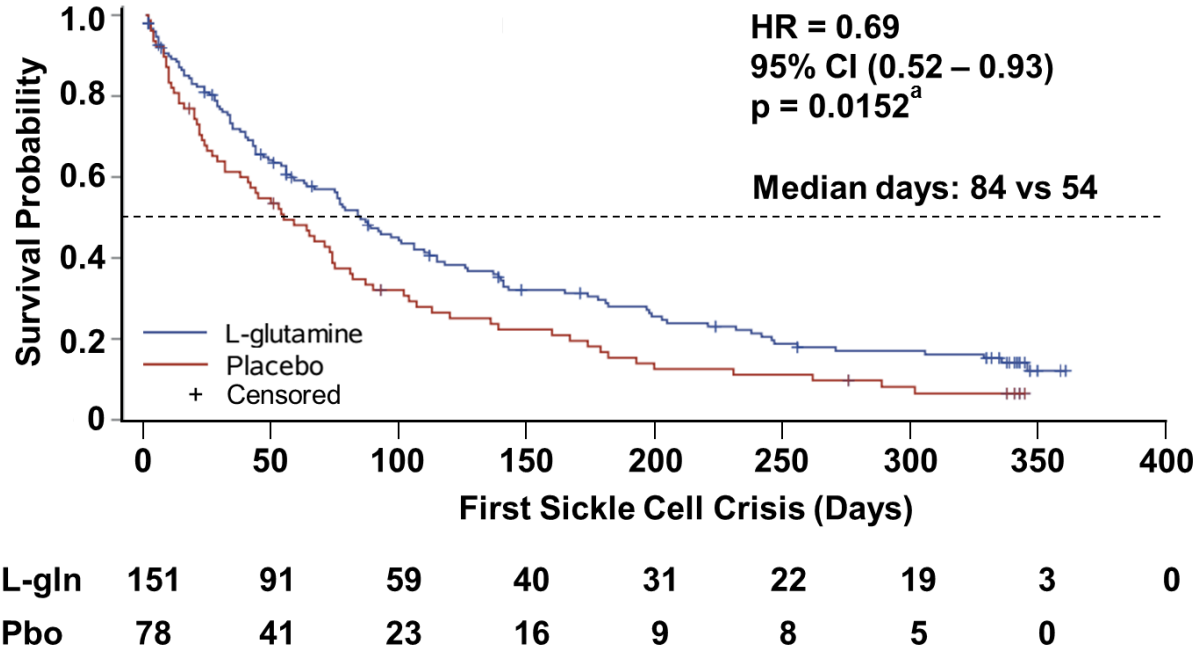
[a] CSR specified endpoint analyzed by CMH using modified ridit scores

[b] 1 patient was randomized but did not take study medication hence was not included in this analysis.

[c] Rate ratio is (rate per 48 weeks for L-glutamine)/(rate per 48 weeks for placebo). A rate ratio < 1 favors L-glutamine.

In addition, a time to first SCC analysis was conducted per the ISE SAP and a time to second SCC analysis was subsequently conduced as a *post hoc* analysis. These analyses provide a between-arm comparison of the rate of SCC events through the hazard ratio estimate without sensitivity to early terminations. For time to first SCC, there was a significant difference between the treatment groups (Figure 2; p = 0.0152). At the 50th percentile level, time to first crisis was 84 days in the L-glutamine group vs 54 days in the placebo group, a difference of 30 days. The hazard ratio for this analysis was 0.69, corresponding to a risk reduction of 31%.

Figure 2. Time to First Crisis: Study 09-01



L-gln	151	91	59	40	31	22	19	3	0
Pbo	78	41	23	16	9	8	5	0	

[a] Log rank test
CI = confidence interval, HR = hazard ratio, L-gln = L-glutamine, Pbo = placebo.

For time to second SCC, there was also significant difference between the treatment groups (p = 0.0260). At the 50th percentile level, time to second crisis (measured from the beginning of the study) was 212 days in the L-glutamine vs 133 days in the placebo group, a difference of 79 days. The hazard ratio for this analysis was 0.68 (Section 5.2.5.1).

The clinical importance of the observed difference in frequency of SCC and time to first SCC relative to placebo was confirmed by differences relative to placebo in number of occurrences of ACS, hospitalizations, days hospitalized, and blood transfusions (Table 3). The mean frequency of ACS was 67% lower in the L-glutamine group relative to placebo. The median number of hospitalizations for sickle cell pain was approximately 33% lower or 1 hospitalization fewer for the L-glutamine group than for placebo. Cumulative days hospitalized was also lower in the L-glutamine group relative to placebo (6.5 vs 11 days, respectively). Patients in the L-glutamine group also had fewer blood transfusion events (1.42 transfusions per patient in the L-glutamine group vs 2.32 in the placebo group).

Table 3. Summary of Study 09-01 Efficacy Results

Descriptive results	SCCs (median)	Time to onset of first crisis (days)	Acute Chest Syndrome (mean)	Hospitalizations (median)	Cumulative Days Hospitalized (median)	Blood transfusion events (mean)
L-glutamine	3	84	0.1	2	6.5	1.42
Placebo	4	54	0.3	3	11.0	2.32
Difference	-1	30 days	-0.2	-1	-4.5	-0.9
% difference	25%	56%	67%	33%	41%	39%
***P*-value[a]**	0.0052[b]	0.0152[c]	0.0028[b]	0.0045[b]	0.022[d]	NA

SCC = sickle cell crisis, NA = not applicable.
[a] P-value for between group difference.
[b] CMH using modified ridit scores.
[c] ANOVA model with treatment as the main effect.
[d] Wilcoxon rank-sum test.

1.4 Supportive Efficacy Data from the Exploratory Phase 2 Study 10478

1.4.1 Study Design

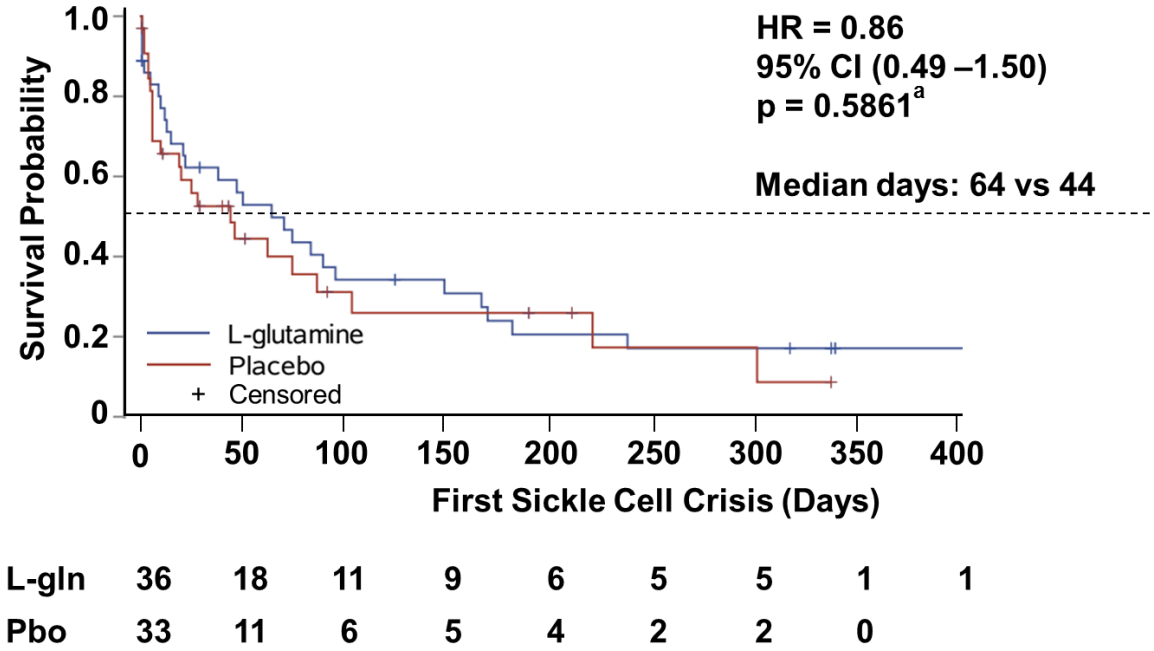
Study 10478 was a Phase 2 randomized, double-blind, placebo-controlled, parallel-group, study to assess the efficacy and long-term safety of oral L-glutamine therapy in patients with sickle cell anemia or sickle β^0-thalassemia who were at least 5 years old. The study design, duration, and inclusion/exclusion criteria in this study were similar to the pivotal Phase 3 Study 09-01. Both studies used the same primary efficacy endpoint, the number of SCCs through Week 48 and prior to the start of taper; however, the definition and methods of classification of an event as an SCC differed between the 2 studies. These changes were implemented to improve the study design for 09-01 based on experience from Study 10478 and to reflect recommendations from FDA (See Section 4.2). The main differences were:

- Although both studies required a visit to a medical facility, Study 10478 required that the medical facility visit last 4 hours or longer. This requirement was not used for Study 09-01.

- In Study 10478, the occurrence of hepatic (liver) sequestration was considered a crisis; while, in Study 09-01 it was not.
- In Study 10478, a programming algorithm was used to determine whether or not an event was considered a crisis; while in Study 09-01, the decision was made by the central adjudication committee.
- In Study 10478, events determined to be crises were not required to be separated by ≥ 24 hours; however, in Study 09-01 events adjudicated as crises were separated by ≥ 24 hours (ie, the difference between the end date of 1 crisis and the start date of the next crisis was required to be at least 2 calendar days).
- Study 10478 utilized a 1:1 randomization scheme compared to 2:1 in Study 09-01.

A trend towards fewer cases of sickle cell crisis was observed in patients treated with L-glutamine in comparison to the placebo group (side by side review in Table 4). Two sensitivity analyses intended to test the effect of imputation methods (last observation carried forward [LOCF] and time-adjusted LOCF) showed the median number of SCCs in the L-glutamine group was 50% lower in the LOCF analysis (1 vs 2 events) and 33% lower in the time-adjusted LOCF analysis (2 vs 3 events) relative to the placebo group. NBR analysis demonstrated a lower rate of SCCs in the L-glutamine treatment group relative to the placebo group ($p = 0.0240$) with a rate ratio of 0.47 (Section 5.3.4). L-glutamine delayed the onset of the first SCC in Study 10478. At the 50th percentile level, time to first crisis was 64 days in the L-glutamine vs 44 days in the placebo group, a difference of 20 days with a hazard ratio of 0.86 (Figure 3). The median number of hospitalizations for sickle cell pain was 50% lower or 1 hospitalization fewer for the L-glutamine group than for placebo (Section 5.3.5.1).

Figure 3. Time to First Crisis: Study 10478



CI = confidence interval, HR = hazard ratio.

Side by side review of data from pivotal efficacy Study 09-01 and the supportive efficacy Study 10478 reveals consistent treatment effects including fewer SCCs per 48 weeks in favor of the L-glutamine treatment group relative to placebo (Table 4).

Table 4. Study 09-01 and Study 10478 Efficacy Results

	Pivotal Study 09-01		Supportive Study 10478	
Endpoints	**L-glutamine N = 152**	**Placebo N = 78**	**L-glutamine N = 37**	**Placebo N = 33**
Primary Endpoint: Number of SCCs using modified ridit scores and CSR imputation rules				
Primary analysis[a]:				
P-value (controlling for region and HU use)	0.0052			
P-value (controlling for center)			0.1501	
Descriptive statistics				
Mean (SD)	3.2 (2.24)	3.9 (2.54)	4.3 (5.22)	9.6 (17.88)
Median (min, max)	3.0 (0, 15)	4.0 (0, 15)	4.0 (0, 27)	4.0 (0, 90)
NBR Analysis - Rate of SCCs Per 48 Weeks				
P-value	0.0374		0.0240	
Rate per 48 weeks	3.25	4.19	4.25	9.07
(95% CI)	(2.76, 3.83)	(3.44, 5.11)	(2.47, 7.32)	(5.35, 15.38)
Rate ratio[b]	0.78		0.47	
(95% CI)	(0.61, 0.99)		(0.24, 0.91)	

CI = confidence interval, CSR = clinical study report, HU = hydroxyurea, NBR = negative binomial regression, SCCs = sickle cell crises, SD = standard deviation.

[a] CSR specified endpoint analyzed by CMH using modified ridit scores.

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

1.4.2 Efficacy Summary

The 5 Legacy studies conducted under an investigator-initiated IND support the biological plausibility of increased glutamine uptake in sickle cell RBCs as a means of altering redox potential in these cells, thereby achieving reduced adherence and, ultimately, a clinical reduction in SCCs. The clinical trends observed in the exploratory study 10478, including fewer SCCs, hospitalizations, and longer time to first SCC relative to placebo, were consistent with these promising results and supportive of continuing on to a Phase 3 clinical trial. In Study 09-01, L-glutamine treatment resulted in a lower frequency of sickle cell painful crises, longer time to first and second crisis, fewer ACS, fewer hospitalizations, fewer days hospitalized, and fewer transfusions relative to placebo. For the primary endpoint, a statistically significant difference between the distribution of crises over the 48 week study period was observed between the L-glutamine and placebo groups (p = 0.0052). A difference of 30 days was observed for median time to first and second crisis in the L-glutamine group relative to placebo with a hazard ratio of 0.69. For time to second crisis (measured from the beginning of the study), a difference of 79 days was observed with a hazard ratio of 0.68. For median hospitalizations, there was a 33% difference in the L-glutamine and placebo groups. Patients in the L-glutamine group also experienced fewer cases of ACS in comparison to placebo by 67%. A post-hoc review of the blood transfusions, as requested by the FDA, showed that the patient in the L-glutamine had fewer episodes of transfusions as compared to placebo. For this rare disease population, with only 1 approved anti-sickling drug for adults and none approved for pediatric use, oral

L-glutamine has established efficacy in both pediatric and adult patients and offers a potential new treatment option with a favorable benefit-risk profile as described below.

1.5 Safety

The L-glutamine clinical development program assessed the safety and tolerability of L-glutamine. Studies 10478 and 09-01 were included in the integrated safety analyses for this submission and are presented as the "Safety Population" in this briefing document. In the pooled analysis of Study 10478 and Study 09-01, the total exposure to L-glutamine was 137.7 patient-years.

Baseline demographics were similar between the L-glutamine and placebo treatment groups. Treatment-emergent adverse events (TEAEs), which include SCCs, were reported in 96.3% of patients in the L-glutamine arm and 97.3% of patients in the placebo arm. Drug related TEAEs were noted in 18.7% of patients in the L-glutamine arm and 13.5% of patients in the placebo arm. Of those, drug related serious adverse events (SAEs) were 1.6% in L-glutamine arm and 2.7% in placebo arm (Table 5). In both groups, adverse events were consistent with the disease population and duration of the study.

The proportion of TEAEs leading to withdrawal was slightly higher in the L-glutamine treatment group (2.7%, 5 patients) compared to the placebo treatment group (0.9%, 1 patient). SAEs leading to study drug discontinuation occurred in 2 patients (1.1%) in the L-glutamine treatment group; 1 of these (0.5%) was considered to be related to study drug. Serious adverse events leading to study drug discontinuation occurred in 1 patient (0.9%) in the placebo treatment group.

Table 5. Overall Summary of AEs (Safety Population)

Parameter	L-glutamine N = 187	Placebo N = 111
Number of events		
Total number of TEAEs[a]	1904	1299
Total number of drug-related TEAEs[b]	77	32
Total number of SAEs	481	411
Total number of drug-related SAEs	5	6
Number of patients with events		
Patients with at least 1, n (%)		
TEAE	180 (96.3)	108 (97.3)
Drug-related TEAE	35 (18.7)	15 (13.5)
SAE	141 (75.4)	89 (80.2)
Drug-related SAE	3 (1.6)	3 (2.7)
Patients who discontinued treatment, n (%)		
Due to TEAE	5 (2.7)	1 (0.9)
Due to drug-related TEAE	3 (1.6)	0 (0.0)
Due to SAE	2 (1.1)	1 (0.9)
Due to drug-related SAE	1 (0.5)	0 (0.0)
Patients who died, n (%)	4 (2.1)	0 (0.0)
Patients who died due to TEAE, n (%)	3 (1.6)	0 (0.0)
Patients who died due to drug-related TEAE, n (%)	0 (0.0)	0 (0.0)

Studies included: Study 10478 and Study 09-01.
AE = adverse event, SAE = serious adverse event, TEAE = treatment-emergent adverse event.
a. A TEAE is defined as an AE with onset date on or after the first dose of study drug and through 30 days after last dose of study medication.
b. Drug-related TEAEs are those with relationship to study drug reported as 'possible', 'probable', 'definite', or missing.

There were 3 deaths due to TEAS in L-glutamine-treated patients. One death occurred in Study 10478 and two deaths occurred in Study 09-01. One additional death was not considered treatment-emergent as it occurred more than 30 days after the last dose of study medication. None of the deaths were considered by the investigator to be related to study drug. No deaths were reported in the placebo group. All patients who died had been suffering serious co-morbidities and were also receiving HU throughout the observation period. Their ages at the time of death were 37, 46 and 45 respectively.

A summary of mortality is presented in Table 6. In this analysis, the crude mortality rate was 1.6% and the exposure-adjusted mortality rate was 2.2 deaths per 100 patient-years. These rates were lower than mortality rates described in published clinical trials that enrolled patients with a history of 2 or more sickle cell crises per year (Steinberg et al, 2003, Ataga et al, 2017).

Table 6. Summary of Mortality (Safety Population)

Parameter	L-glutamine (N = 187)
Number of treatment-emergent deaths	3
Crude mortality (%)	1.6
Total exposure in patient-years	137.7
Mortality per 100 patient-years	2.2

Crude mortality = (number of treatment-emergent deaths/number of patients in each group) × 100.
Total exposure in patient-years = summation of all exposure/365.25, where exposure = last dose date – first dose date + 1.
Mortality per 100 patient-years = number of deaths/total exposure in patient-years × 100.

Post marketing data and literature reports for NutreStore® (L-glutamine for the treatment of short bowel disease) were also evaluated from 01 Aug 2008 to 10 Jun 2016. Over this time period, approximately 284 courses of treatment were distributed. No adverse events were reported over this postmarketing period.

The clinical development program established the safety of L-glutamine 0.3 gram/kg twice daily (bid) for up to 48 weeks in adult and pediatric patients. Serious events were common in both treatment groups and consistent with the underlying disease. Overall, L-glutamine was well tolerated with a safety profile similar to placebo.

1.6 Benefit/Risk Summary

Sickle cell disease is a devastating, rare, hereditary disease associated with profound clinical manifestations, including premature mortality and shortened lifespan. The organ damage caused by each crisis is cumulative, and patients who have a higher frequency of crises have a higher fatality rate.

While treatment with HU has a positive benefit/risk ratio in some adult SCD patients, many will continue to experience SCCs at a frequency that places them at considerable risk for organ damage and early death. In the US, there are no approved treatments for SCD in pediatric patients. There is a well-documented need for therapies that can further reduce the frequency of SCCs in both pediatric and adult patients.

Pre-clinical studies demonstrated that glutamine supplementation significantly improves NAD redox potential, thereby establishing the biological plausibility of L-glutamine in the treatment of SCD. The clinical trends observed in the earlier Phase 2 Study 10478, including fewer SCCs, hospitalizations, and longer time to first SCC relative to placebo, were consistent with these promising results and supportive of the later findings from the Phase 3 clinical trial.

In Phase 3 Study 09-01, L-glutamine treatment resulted in a statistically significant difference in the distribution of the number of SCCs relative to the placebo group (p=0.0052). The L-glutamine group also experienced lower frequencies of ACS and, hospitalization, with median

differences of 67% and 33%, respectively. Of note, these results were achieved in a patient population where two thirds of the patients were also taking HU.

L-glutamine was well tolerated. The integrated safety data demonstrated that oral L-glutamine at 0.3 gram/kg bid had a safety profile similar to placebo in both adults and children. Although not considered to be treatment related by the investigators, there were 3 treatment-emergent deaths in L-glutamine-treated patients and none in the placebo group. The crude mortality rate was 1.6% and the exposure-adjusted mortality rate was 2.2 deaths per 100 subject-years. These rates were lower than mortality rates described in recently reported clinical trials that enrolled patients with a history of 2 or more sickle cell crises per year (Steinberg et al, 2003, Ataga et al, 2017).

The difference in the number of SCCs relative to placebo, longer time to first and second crisis, and associated clinical benefits of reduced hospitalization, cumulative days in hospital, frequency of acute chest syndrome, and fewer blood transfusion events are clinically meaningful benefits in a vulnerable patient population. When combined with a very favorable safety profile, L-glutamine clearly provides a positive benefit/risk assessment for both pediatric and adult SCD patients.

2 PRODUCT DEVELOPMENT RATIONALE

2.1 Sickle Cell Disease

Sickle cell disease is an autosomal recessive disease caused by a single point mutation (GAG to GTG) in the β^A globin gene that results in the formation of abnormal hemoglobin (HbS). This physically alters deoxygenated hemoglobin, causing polymerization of the molecule within RBCs. These hemoglobin polymers reduce the deformability of RBCs, leading to increased rigidity and formation of the characteristic sickle shape (Steinberg, 2011). The cell membranes of sickle cells are porous and easily dehydrated, with abnormal flow properties due both to their shape and increased exposure of adhesive ligands on the surface of the cell. These abnormal cells interact with other RBCs in circulation and with vascular endothelial cells to trigger vaso-occlusion and the ensuing clinical symptoms of SCD (Hebbel et al, 1980).

A devastating and rare hereditary disease associated with profound clinical manifestations and shortened lifespan, SCD is an inherited blood disorder. Approximately 100,000 Americans suffer from SCD in the US. Most of those affected by SCD are of African ancestry or self-identify as Black while a minority are of Hispanic or southern European, Middle Eastern, or Asian Indian descent. Among African Americans the incidence of SCD at birth is approximately 1 in 365, while the incidence among Hispanic-Americans at birth is approximately 1 in 16,300 (CDC). The disease is associated with major morbidity and the lifespan of children with SCD is shortened by 2 or 3 decades compared to the general population. Among the children with Hb SS disease (sickle cell anemia), 1% died as a result of SCD-related causes during the first 3 years of life (Platt et al, 1994).

Treatment of sickle cell crisis is burdensome and expensive for patients and public payers, as it encompasses costs for hospitalization, ER visits, urgent care visits, and prescription pain medication (Steinberg, 2011). According to an article in American Journal of Hematology, "The Burden of Emergency Department Use for Sickle Cell Disease: An Analysis of the National Emergency Department Sample Database" by Lanzkron et al (October 2010), there were approximately 70,000 hospitalizations and 230,000 ER visits by SCD patients in 2006, with combined ER and hospital inpatient charges estimated to be $2.4 billion. In another study of Healthcare Cost and Utilization Project (HCUP) State Inpatient Databases and State Emergency Department Databases using 2005 and 2006 data, the 30-day re-hospitalization rate of SCD patients was 33.4% (95% confidence interval [CI], 33.0% - 33.8%); the 14-day re-hospitalization rate was 22.1% (95% CI, 21.8% - 22.4%) (Brousseau et al, 2010). Hospital stays for SCD patients are largely billed to public payers, with 66% paid by Medicaid and 13% paid by Medicare between 1994 and 2004 (Steiner, 2006).

2.2 Sickle Cell Disease Complications

The most common complication of SCD is repeated bouts of SCCs of varying frequency, often severe enough to require hospitalization (Steinberg, 2011). These acute, episodic vaso-occlusive crises result in painful ischemia (manifested by acute pain and tenderness, fever, tachycardia, and anxiety) and may cause organ failure, accounting for most of the morbidity associated with SCD (Kasper et al, 2005). The pain associated with these events is typically rapid in onset and can be

excruciatingly severe, persisting for several days and requiring management with both long- and short-acting opioids (Steinberg, 2011). If patients recover between SCCs, they often resume a relatively normal life; however, the frequency of SCCs is not predictable. Some patients have few painful events, while others may require hospitalization several times a year. Other patients may go months without an SCC and then experience a cluster of severe attacks.

While frequency of SCCs varies among patients, even a single crisis is considered a significant event due to the severity of the pain, impact on quality of life, and associated decrease in survival (Platt et al, 1991; Hillman et al, 2011). Importantly, the overall survival of patients in the U.S. with sickle cell anemia is correlated with disease state severity as measured by number of SCCs experienced by patients annually. Patients who experience more than 3 crises per year are more likely to experience fatal complications during their 30's and 40's in comparison to a median survival of nearly 50 years in patients who experience between 1 and 3 crises per year (Hillman et al, 2011). Among Black Americans with SCD, SCC can lead to multi-organ damage and early death with a decrease in life expectancy of 25 to 30 years in comparison to the Black American population in general (Platt et al, 1994).

Acute chest syndrome is the second most common reason for hospitalization in SCD, affecting more than half of all patients (Steinberg, 2011). ACS is characterized by fever, chest pain, cough, and lung infiltrates. Treatment of ACS is managed through transfusions, antibiotics, hydration with careful avoidance of over hydration, respiratory therapy with bronchodilators, incentive spirometry, and maintenance of tissue oxygenation. Oxygen is used when the patient is hypoxic or tachypneic and has signs of respiratory distress. Opioid use should balance pain relief with the danger of respiratory suppression. Long-term opioid use is common and a major problem, as it can induce physiologic tolerance and reduce efficacy. Treatment of acute episodes can become difficult and interruption of use can lead to withdrawal syndromes characterized by pain.

Aside from ACS, major causes of morbidity and mortality in SCD patients are pulmonary disease in adults and infection in children. Other complications include anemia, retinopathy, leg ulcers, priapism, renal disease, digestive system disease, and neurocognitive dysfunction. Chronic complications of SCD can affect almost any organ, and certain acute complications, such as stroke and priapism, often evolve into chronic phases that require special approaches to management.

2.3 Current Sickle Cell Disease Treatment Options

SCC is a serious and potentially life-threatening consequence of SCD and lowering the frequency of these events remains a major unmet need in SCD patients. Currently, SCD treatment is largely focused on disease and pain management, treatment of complications, and acute care during sickling crises (Steinberg, 2011). Treatment approaches for SCD include HU, blood transfusion, and stem cell transplantation.

Hydroxyurea is the only drug approved by the FDA to reduce the frequency of SCCs and to reduce the need for blood transfusions in adult SCD patients. HU reduces, but does not eliminate SCCs. Many patients treated with HU will continue to experience SCCs at a frequency that

places them at considerable risk for organ damage and early death. In addition, some patients cannot tolerate treatment with HU due to adverse effects such as leukopenia, neutropenia, anemia, and thrombocytopenia (Droxia® USPI, 2016). Thus, in adult SCD patients there is an unmet need both for treatments that can be taken in combination with HU in order to further reduce the frequency of SCCs, and for treatments that are effective in patients who cannot tolerate HU.

There are currently no approved therapies for reduction of SCCs in children with SCD. This is an area of critical need because the symptoms and organ damage of SCD begin within the first years of life. Additionally, approximately 1 in 10 patients with SCD will have clinically apparent strokes before the age of 20 (Verduzco and Nathan 2009). Silent strokes are also prevalent and are associated with brain damage and cognitive impairment. This clearly supports an urgent need for treatments to reduce SCCs that can be started early in life, before the development of irreversible vasculopathy and organ damage.

Blood transfusions are sometimes used to reduce sickle cell hemoglobin levels. Simple transfusions only require peripheral venous access and are rapidly available but reduce HbS levels gradually, while exchange transfusions take more time to initiate and require more complicated venous access but are able to reduce HbS levels much more rapidly. However, transfusions have a potential for alloimmunization, cause hyperviscosity, and transmit infectious agents. They can also lead to abnormally high levels of iron in the blood, which can cause long-term organ damage and adverse reactions due to a mismatch between the donors and recipients. For these reasons, transfusions are not appropriate to manage a routine painful crisis (Steinberg, 2011). Myeloablative stem cell transplantation has a high mortality rate (about 5%) and a high rate of rejection or disease recurrence (10%), limiting the use of this procedure to children with severe disease.

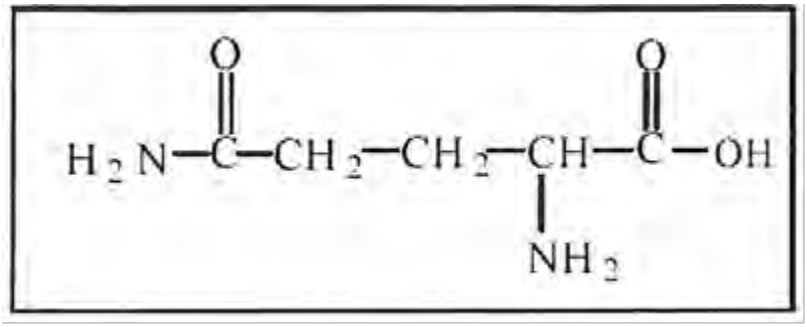
Taken together, although treatment options and symptom management techniques do exist for SCD patients, the severity of pain episodes, lack of effective treatment for children, potential of acute and chronic side effects, and rising costs of managing SCD for both patients and public payers are strong indicators of an unmet need for additional treatments to reduce the frequency of SCCs.

3 L-GLUTAMINE BACKGROUND

3.1 Product Description

The chemical name of the product is (S)-2-aminoglutaramic acid, L-glutamic acid 5-amide, (S)-2,5-diamino-5-oxopentanoic acid, or L-glutamine. The molecular formula of L-glutamine is $C_5H_{10}N_2O_3$. The structural formula is shown below in Figure 4.

Figure 4 Structural Formula



3.2 L-Glutamine Proposed Indication, Dosing and Administration

The sponsor is seeking approval of L-glutamine for the indication of the treatment of SCD in adult and pediatric patients. The proposed dose of L-glutamine powder for oral solution is 0.3 gram/kg of L-glutamine in increments of 5 gram/dose (10 gram/day) based on weight, with an upper limit of 30 gram/day, administered orally twice daily (Table 7). The Phase 2 and Phase 3 clinical trials treated patients up to 48 weeks. L-glutamine is intended for long-term use in patients with SCD.

Table 7. Oral L-glutamine Proposed Dosing

Weight in Kgs	Weight in lbs	Per dose in grams	Per day in grams	Packets per dose	Packets per day
< 30	< 66	5	10	1	2
30 - 65	66 - 143	10	20	2	4
> 65	> 143	15	30	3	6

3.3 Development of Oral L-glutamine for Treatment of SCD

L-glutamine is one of the most ubiquitous amino acids, with a high utilization rate in comparison to most other amino acids (Wernerman and Hammarqvist, 1994). Although the human body can synthesize L-glutamine, during stress or severe illness, an exogenous supplement is often required due to an increased demand. This makes L-glutamine a conditionally essential amino acid (Shabert and Ehrlich, 1994). Aside from being a building block for protein, a preferred fuel for rapidly dividing cells including hematopoietic cells (Smith, 1990a), and a precursor for

glutathione (Yoshida et al, 1995), glutamine also serves as a precursor of nucleic acids and nucleotides including the pyridine nucleotides, NAD and NADH (Smith and Wilmore, 1990b).

These pyridine nucleotides play key roles in the regulation and prevention of oxidative damage in RBCs, as evidenced by conditions such as methemoglobinemia where inhibition or deficiency of an NADH-dependent enzyme slows the conversion of methemoglobin to hemoglobin (Jaffe, 1974). 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 RBC (Asakura et al, 1977; Campwala and Desforges, 1982; Chiu et al, 1979; Das and Nair, 1980; Hebbel et al, 1982; Jain and Shohet, 1984). This increased susceptibility to oxidation of sickle RBCs may contribute to chronic hemolysis (Bensinger and Gillette, 1974) and vaso-occlusive events in SCD (Hebbel et al, 1982). In addition, sickle RBCs were found to have high NAD levels accompanied by a decrease in NAD redox potential (defined by the ratio of NADH to total NAD), 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 et al, 1988).

In vitro analyses of L-glutamine transport in RBCs from individuals with sickle cell anemia showed an approximately 3-fold increase compared to RBCs from individuals with high reticulocyte counts. In addition to a higher affinity for and enhanced transport of L-glutamine in sickle RBCs, there is enhanced conversion of actively transported L-glutamine to glutamate (a byproduct of L-glutamine in NAD synthesis) compared to controls (Niihara et al, 1997). As the Michaelis-Menten constant (Km) of glutamine for NAD synthetase is significantly higher than the intracellular concentration of glutamine (Zerez et al, 1990), the increase of L-glutamine concentration in the intact sickle RBC is thought to further increase the rate of NAD synthetase activity. The biological plausibility of L-glutamine in the treatment of SCD is further summarized by the following:

- In vivo analyses demonstrated that glutamine supplementation improved NAD redox potential and resulted in a positive subjective clinical response (Niihara 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 et al, 1996).
- L-glutamine at a dose of 30 grams daily for at least 4 wks significantly decreased endothelial cell adhesion in sickle RBCs compared to untreated sickle RBCs in a static human umbilical cord model (Niihara et al, 2005). In all patients treated with L-glutamine, there was large decrease in adhesion rate ($p < 0.001$).

Collectively, these data suggested that L-glutamine may provide a clinically protective effect via increased RBC deformability and decreased cell adhesion.

The clinical development program evaluating L-glutamine for the treatment of SCD began with Investigator initiated trials (legacy studies) conducted with support from the NIH. Legacy studies are summarized below.

- **Study 8288** was a 4 week study in which a total of 7 adult SCD patients received oral L-glutamine at a daily dose of 30 grams. In this study, significant changes in both the

NADH level and NAD redox potential were observed in SCD patients suggesting that L-glutamine may decrease the oxidative susceptibility of sickle cell RBCs and result in clinical benefit.

- **Study 8822** was a 4 week dose finding study in which a total of 11 adult SCD patients received oral L-glutamine at daily doses of 10, 20, and 30 grams. In the 30 gram/day dose group, there was a consistent increase in mean NADH and NAD redox potential suggesting that the 30 gram/day dose would be optimal for clinical trials in patients with sickle cell anemia.
- **Study 8775** was 60 week early Phase 2 prospective randomized double blind crossover clinical trial to examine the efficacy of oral L-glutamine therapy for sickle cell anemia. L-glutamine 30 gram/day or placebo was administered orally as 10 grams, 3 times/day, to patients for 24 weeks. A total of 24 eligible patients were enrolled in the study and 6 completed and were evaluable. A trend toward improvement in the number of painful crises was observed.
- **Study 10511** was a 12 week prospective, randomized, double-blind, placebo-controlled parallel-group, single-center study to examine the effect of L-glutamine therapy on exercise endurance and breath in patients with SCD. Doses were calculated by weight and the upper limit for the daily of L-glutamine was 30 grams. A total of 15 patients were evaluable in the study, 5 in the L-glutamine group and 10 in the placebo group. There were no notable differences in AEs and SAEs between the 2 groups.
- **Study 10799** was a 12 week open-label study to evaluate the effect of L-glutamine treatment on the consistent improvement in the patient's subjective perception of clinical status, safety, and exercise endurance. A total of 14 patients with SCD and 5 control patients received 30 gram/day of L-glutamine administered orally in 2 divided doses for 12 weeks. Six sickle cell anemia patients completed they study and were analyzed. The results suggested that L-glutamine therapy improved the exercise endurance of sickle cell anemia patients.

Based on the promising results of the legacy studies, a Phase 2 study, Study 10478, and pivotal Phase 3 study, Study 09-01, were conducted to evaluate the efficacy and safety of L-glutamine in SCD patients (Section 5). Along with these clinical milestones, L-glutamine was granted an orphan drug designation for the treatment of SCD in 2001, and a Fast Track designation in 2005. Throughout clinical development, Emmaus has worked closely with the FDA to agree upon the supportive non-clinical pharmacology and toxicology data, confirm the clinical pharmacology knowledge, confirm the suitability of the design and dosing in the Phase 2 and Phase 3 studies, and discuss the statistical analysis of the Phase 3 study. Details of these regulatory interactions are summarized in (Section 4).

4 L-GLUTAMINE CLINICAL DEVELOPMENT AND REGULATORY HISTORY

The L-glutamine clinical development program represents a comprehensive evaluation of the safety and efficacy of SCD that is appropriate in scope for a rare and serious condition with significant unmet need. The program consists of 7 studies conducted in patients with SCD. Five of these studies were exploratory clinical trials conducted to characterize L-glutamine-mediated decreases in oxidative stress in RBCs, and its role in reducing deformability and adherence of sickle RBCs to endothelial cells in patients with SCD (Table 8). The majority of SCD patients evaluated were enrolled in Phase 2 Study 10478 (23 Apr 2004 to 29 May 2008) and Phase 3 Study 09-01 (21 Jun 2010 to 19 Dec 2013). These 2 randomized, placebo-controlled clinical trials evaluated the long-term safety and efficacy of L-glutamine in the treatment of SCD in adult and pediatric patients (Table 9). From these 2 studies, the Safety Population was derived with a total of 187 of 298 patients exposed to L-glutamine for a mean duration of 268.9 days. The majority of patients in both treatment groups received at least 48 weeks of treatment (58.3% in the L-glutamine treatment group and 65.8% in the placebo treatment group). In the L-glutamine and placebo treatment groups, respectively, 7.0% and 9.0% of patients received ≥ 53 weeks of treatment.

Table 8. Legacy Clinical Trials Evaluating Oral L-Glutamine

Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Dosage regimen	Number of Patients	Healthy Patients or Diagnosis of Patients	Duration of Treatment
8288 (Niihara, 1998)	To evaluate the effect of L-glutamine treatment on total NAD, NADH, and NAD redox potential of sickle RBCs, as well as hematologic parameters, subjective clinical response, and safety of L-glutamine supplementation	Pilot study, open-label, uncontrolled	L-glutamine 10 g/day; tid; Oral	7	Patients with sickle cell anemia (homozygous Hb SS)	4 weeks
10779	To evaluate the effect of L-glutamine treatment on the consistent improvement in the patients' subjective perception of clinical status (especially energy levels), safety, and exercise endurance	Open-label, controlled	L-glutamine 30 g/day (divided in 2 doses),	L-glutamine: 14 control: 5	Patients with sickle cell anemia (homozygous Hb SS) or healthy patients	12 weeks
10511	To evaluate the effect of L-glutamine treatment on exercise endurance, breath by breath exercise response, the incidence of painful crises, level of chronic pain, amount of daily requirement of narcotics, and safety	Phase 2, prospective, randomized, double-blind, placebo-controlled, parallel-group	L-glutamine 0.3 g/kg/day or placebo; bid; Oral	L-glutamine: 5 placebo: 10	Patients with sickle cell anemia or sickle β^0-thalassemia	12 weeks
8775	To evaluate the effect of L-glutamine treatment on total NAD, NAD redox potential, RBC endothelial adhesiveness, hematologic parameters, frequency of painful crises, no. of hospitalization days, no. of painless days on study, and safety	Phase 2a, prospective randomized double-blind, placebo-controlled, crossover	L-glutamine 10 g/day or placebo; tid; Oral	24	Patients with sickle cell anemia (homozygous Hb SS)	53 weeks
8822	To evaluate the effects of 3 different daily doses of oral L-glutamine on change from baseline in NADH, NADH/NADT redox potential, and hematological parameters	Open-label, uncontrolled	L-glutamine 10 g/day, 20 g/day and 30 g/day; Oral	10 g/day: 6 20 g/day: 4 30 g/day: 7	Patients with sickle cell anemia (homozygous Hb SS)	4 weeks

14 C-L-Glutamine = carbon-14-labeled glutamine, bid = twice daily, Hb SS = homozygous hemoglobin SS type, Km = Michaelis Menten Constant, NAD = nicotinamide adenine dinucleotide, NAD+ = oxidized nicotinamide adenine dinucleotide, NADH = reduced nicotinamide adenine dinucleotide, NAD redox potential = ratio of NADH to total NAD, total NAD = NAD+ + NADH, RBC = red blood cell, tid = three times daily.

Table 9. Studies Evaluating the Efficacy of Oral L-Glutamine

Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Dosage regimen	Number of Patients	Healthy Patients or Diagnosis of Patients	Duration of Treatment
10478	To evaluate the effect of L-glutamine treatment on the occurrences of painful sickle cell crises, frequency of hospitalizations and ER visits, and safety	Phase 2, prospective, randomized, double-blind placebo-controlled, parallel-group	L-glutamine 0.3 g/kg/day or placebo; bid; Oral	L-glutamine: 37 placebo: 33	Patients with sickle cell anemia or sickle β^0-thalassemia	51 weeks
09-01	To evaluate the effect of L-glutamine treatment on the occurrence of sickle cell crises, frequency of hospitalizations and ER/medical facility visits, and safety	Phase 3, prospective, randomized, double-blind, placebo-controlled, parallel-group	L-glutamine 0.3 g/kg or placebo; bid; Oral	L-glutamine: 152 placebo: 78	Patients with sickle cell anemia or sickle β^0-thalassemia	51 weeks

ER = emergency room.

4.1 Clinical Pharmacology

Pilot Study in Patients With SCD (Study 8288)

Study 8288 was an open-label, uncontrolled, single center pilot study to evaluate the clinical and biochemical effects of oral L-glutamine in adult patients with SCD. Patients were evaluated at baseline, weekly or biweekly and after 4 weeks of treatment with L-glutamine. Focused interviews were conducted on chronic pain, energy level, usage of narcotics and activity levels. Extracts from whole blood were assayed for NAD and NADH using spectrophotometric enzymatic cycling assays. The objectives of the study were to evaluate the effects of oral L-glutamine on hematologic parameters, NAD, NADH, and subjective clinical response.

Eligible patients were adults (19 to 60 years) with a diagnosis of sickle cell anemia and homozygous for Hb SS. Patients were excluded from the study if they were pregnant, had received a transfusion of a blood product in the previous 3 months or were currently taking or had previously received treatment for SCD with HU. A total of 30 gram/day L-glutamine was administered orally as 10 grams, 3 times/day to patients for 4 weeks.

A total of 7 patients were enrolled into the study and all patients completed 4 weeks of treatment. There was a statistically significant increase in the mean NADH level at 4 weeks with a resulting increase in NAD redox potential (ratio of NADH to total nicotinamide adenine dinucleotide [NADT]). Table 10 summarizes the key results at baseline and after 4 weeks of treatment.

Table 10. NADH, Total NAD, Redox Potential and Hemoglobin at Baseline and 4 weeks

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

Values presented are mean (SD) and range.
NAD = nicotinamide adenine dinucleotide, NADH = nicotinamide adenine dinucleotide hydride, NS = not significant, RBC = red blood cell.

Hemoglobin levels did not change significantly after 4 weeks of treatment with L-glutamine. Subjective clinical responses suggested an improvement over the 4 week treatment period (Niihara et al, 1998).

This small proof of concept study demonstrated that 30 gram/day of L-glutamine administered over 4 weeks significantly increased NADH and NAD redox potential from baseline values.

Dose Finding Study in Patients with SCD (Study 8822)

Study 8822 was an open-label, single-center, sequential study that was designed to evaluate 3 daily doses of L-glutamine (10, 20, and 30 gram/day) in adult patients with SCD. Patients were evaluated at baseline, and then after 2 and 4 weeks of treatment with L-glutamine. The objectives of the study were to evaluate the effects of 3 different daily dose levels of oral L-glutamine on change from baseline in NADH, NADH/NADT redox potential, and to obtain subjective clinical response.

Patients were administered either 10 gram/day (N = 6), 20 gram/day (N = 4), or 30 gram/day (N = 7) of L-glutamine for a total of 4 weeks.

There were no significant changes observed in the mean total NAD and NADH levels at 4 weeks in the 10 gram/day and the 20 gram/day dose groups. As observed in the pilot study, in the 30 gram/day dose group, there was a consistent increase in mean NADH and NAD redox potential suggesting that the 30 gram/day dose would be optimal for clinical trials in patients with sickle cell anemia.

4.2 Regulatory History

4.2.1 Indication: Short Bowel Syndrome

L-glutamine was approved and marketed under NDA 21,667 as NutreStore® (L-glutamine powder for oral solution) for the treatment of SBS in patients receiving specialized nutritional support when used in conjunction with a recombinant human growth hormone (also approved for this indication) on 10 Jun 2004.

4.2.2 Indication: Sickle Cell Disease

An investigator-initiated IND application for L-glutamine was originally filed on 15 May 1997 by Yutaka Niihara, MD, MPH. The IND was transferred to the current sponsor, Emmaus Medical, on 02 Aug 2011 (the FDA acknowledged the name change on 17 Feb 2012).

During the time period from initiation of the IND to present, Emmaus interacted with the FDA multiple times. These communications and meetings sought to request guidance and support throughout the development program regarding the indication for the treatment of SCD. The FDA's advice was incorporated into the design and dosing of the Phase 2 and Phase 3 studies, as well as into the statistical analysis of the Phase 3 study. Key interactions during this period include:

- **01 Aug 2001:** L-glutamine was granted an orphan drug designation for the treatment of SCD by the FDA Office of Orphan Products Development.
- **19 Nov 2001:** A meeting was held to gain understanding of the NDA processes/requirements and FDA expectations, including guidances, data management, labeling requirements, and good manufacturing practices. The meeting generated several comments and questions from the FDA, which were addressed in subsequent interactions.

The FDA also provided several recommendations regarding study design and primary/secondary endpoints for Phase 2 study 10478.

- **07 Jan 2005:** Fast-Track designation was granted for the indication to reduce painful crises in patients with SCD.
- **10 Jul 2006:** Correspondence was received from the FDA in response to information provided by Emmaus to address outstanding items from prior meetings and submissions. In this correspondence, the FDA confirmed the acceptability of non-clinical pharmacology and toxicology information presented by Emmaus and addressed the concerns raised at the 19 Nov 2001 meeting. The FDA also noted that the dosing regimen in Study 10478 was acceptable. As a result, the dosing regimen from Study 10478 was continued in Phase 3 study 09-01.
- **20 Apr 2009:** A meeting was held to discuss the design of Phase 3 study 09-01 including: a 48-week duration of treatment, use of the ITT population for analysis, increased sample size, 2:1 randomization, stratification by region and HU usage.
- **06 Jan 2010:** An advice/information letter from FDA was received requesting additional information regarding the clinical and statistical design of Study 09-01. A response was provided to the FDA on 28 Jan 2010 to describe how Emmaus incorporated these recommendations into the design of study 09-01. To address the clinical advice, Emmaus revised the protocol to ensure closely monitored compliance, evaluation of all randomized patients until the end of study, and development of an independent central adjudication committee to evaluate all SCCs. To address the statistical advice, Emmaus stated it would use the CMH test with modified ridit scores (in SAS®) to remain consistent with the method used for sample size calculation and data analysis.
- **05 Nov 2012:** A meeting was held to obtain feedback on the 24-week Interim Analysis Report of Study 09-01 and for guidance on the appropriate next steps including whether to increase the number of patients to be enrolled in the study. At the time, all but one team member were blinded to the results. Emmaus followed the recommendations made by the FDA to refrain from enrolling more patients in the study and to complete the study as planned to its 48-week treatment duration.
- **11 Jun 2014:** A meeting was held to obtain the FDA's feedback on the proposed NDA plan in order to ensure acceptability of review. Preliminary findings from Study 09-01 were presented and discussed. Emmaus followed the recommendation made by the FDA to submit integrated data sets for efficacy and safety.
- **15 Oct 2014:** A meeting was held to discuss the analysis of the completed Phase 3 Study 09-01. Emmaus followed the recommendations made by the FDA to perform additional analyses to assess the compliance role in the efficacy results and sensitivity analysis to handle missing data.

- **07 Sep 2016:** Submission of the NDA for oral L-glutamine for the treatment of sickle cell disease.

5 EFFICACY OF L-GLUTAMINE FOR THE TREATMENT OF SCD

This section presents the results of the Phase 2 and 3 clinical studies supporting the efficacy of L-glutamine in SCD. It begins with a comprehensive review of the pivotal Phase 3 trial design (Section 5.1) and results (Section 5.2), followed by an abbreviated discussion of the Phase 2 study results (Section 5.3), and the overall efficacy conclusions (Section 5.4).

5.1 Pivotal Efficacy Study 09-01

Study 09-01 was a Phase 3 randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the safety and efficacy of oral L-glutamine therapy for patients who were at least 5 years of age with sickle cell anemia or sickle β^0-thalassemia ($\geq$ 2 episodes of painful crises within the 12 months prior to the screening visit). The study consisted of a 4-week screening period, a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period. Patients were randomized in a 2:1 ratio (L-glutamine versus placebo) and stratified by HU usage and by region. Concomitant HU use was permitted in the study as was the use of blood transfusions.

An equivalent volume of oral powder, L-glutamine or placebo, was administered at a dosage of 0.3 gram/kg of patient body weight, twice daily for 48 weeks, with an upper limit of 30 gram/day for patients (Table 11). The dosage was in increments of 5 grams based on weight and the total daily dose was 10, 20, or 30 grams based on weight. After 48 weeks of treatment, the dose was tapered to zero over a 3 week period. Patients were weighed at each study visit and if a patient's weight change was maintained over 2 consecutive study visits, the study drug dosage was adjusted accordingly.

Table 11. Study Drug Dose by Patient Weight – Study 09-01

	Patient Weight Range (kg)	Total Daily Dose (g/day)
Study 09-01	< 30	10
	30 - 65	20
	> 65	30

5.1.1 Patient Population

To be eligible, a patient had to be at least 5 years old with documented diagnosis of sickle cell anemia or sickle ß^0-thalassemia by hemoglobin electrophoresis with at least 2 crises in the year prior to screening (Table 12). There was no upper limit on the number of crises. If the patient had been treated with HU prior to the Screening Visit, the therapy must have been continuous and stable for at least 3 months with the intent to continue for the duration of the study. The patient or the patient's legally authorized representative must have given written informed consent and an assent was also obtained when applicable. If the patient was a female of child-bearing potential, she agreed to practice a recognized form of birth control during the course of the study.

Table 12. Inclusion Exclusion Criteria – Study 09-01

Inclusion criteria

- ≥ 5 years of age
- Diagnosed with sickle cell anemia or sickle β^0-thalassemia (documented by hemoglobin electrophoresis)
- ≥ 2 episodes of painful crises within 12 months of the screening visit
- Therapy with an anti-sickling agent within 3 months of the screening visit must have been continuous for at least 3 months with the intent to continue for the next 14 months or for the duration of the study
- Informed consent given by patient or the patient's legally authorized representative
- Agreed to practice a recognized form of birth control during the course of the study (if the patient was a female of childbearing potential)

Exclusion criteria

- Significant medical condition that required hospitalization (other than sickle painful crisis) within 2 months of the screening visit
- Prothrombin time INR > 2.0
- Serum albumin < 3.0 g/dL
- Received any blood products within 3 weeks of the screening visit
- History of uncontrolled liver disease or renal insufficiency
- Patient was pregnant or lactating[a]
- Treated with any form of glutamine supplement within 30 days of the screening visit
- Treated with an experimental anti-sickling medication/treatment (except HU) within 30 days of the screening visit[b]
- Treated with an investigational drug within 30 days of the screening visit[c]
- Enrolled in an investigational drug or device study and/or had participated in such a study within 30 days of the screening visit
- Factors that would have made it difficult for the patient to comply with the requirements of the study (in the judgment of the investigator)

HU = hydroxyurea, INR = international normalized ratio.

[a] Patients that had the intention of becoming pregnant during the study were also excluded.

[b] The exception of HU only applied to pediatric patients.

[c] This criterion did not apply to HU use in pediatric patients.

5.1.2 Measurement of Treatment Compliance

Patients were dispensed a 5-week supply of study medication at each study visit and treatment compliance was measured by recording used and unused study medication. Patients were also asked questions regarding study medication intake on their patient daily diary card. Compliance was measured by reviewing the number of days patients were on the study and the number of uninterrupted days dosed prior to the start of taper, and the results were summarized for each treatment group. Compliance was also evaluated in terms of the percentage of study medication taken during participation in the treatment period.

5.1.3 Endpoints for Efficacy Evaluation

The primary objective in Study 09-01 was to demonstrate a difference in the number of SCCs in the group of patients receiving L-glutamine compared to those receiving placebo. The primary

efficacy endpoint was the number of SCCs through Week 48 and prior to start of taper. The definition of an SCC in this study is provided in Section 5.1.4.3.

The efficacy measurements utilized are widely used and generally recognized as reliable, accurate, and clinically meaningful. The primary and additional endpoints presented in this submission are summarized in Table 13.

Sickle cell crises were recorded on the adverse event case report forms and reviewed by an independent central adjudication committee (CAC) for inclusion in the efficacy analysis. (See Section 5.1.4.3 for details). All investigator-reported adverse events, including those adjudicated as SCCs, were included in the safety summaries.

Additional efficacy endpoints specified in the ISE included time to first crisis, occurrences of ACS, number of hospitalizations for sickle cell pain, percentage of time hospitalized, number of ER visits for sickle cell pain, and hematologic parameters. Time to second crisis and frequency of blood transfusions were also evaluated as *post hoc* analyses.

Table 13. Efficacy Endpoints

Type of Endpoint	Endpoint
Primary efficacy	Number of SCCs
Additional efficacy	Number of hospitalizations for sickle cell pain
	Number of ER visits for sickle cell pain
	Occurrences of ACS
	Time to first crisis
	Percentage of time hospitalized
	Hematologic parameters

ACS = acute chest syndrome, ER = emergency room, SCCs = sickle cell crises.
Hematologic parameters included hemoglobin, hematocrit, and reticulocyte counts.

5.1.4 Statistical Methods

5.1.4.1 Determination of Sample Size

The sample size required for the study was calculated to be 220, with 147 patients assigned to L-glutamine therapy and 73 patients assigned to placebo. The study was expected to have a 25% dropout rate, with an estimated 110 patients on L-glutamine completing the study and 55 patients on placebo completing the study; the number of completed patients in each treatment group provides 80% power to detect a difference between the groups in the distribution of the number of sickle-cell crises at Week 48. Sample size calculation was based on a significance level of 0.048, and power was calculated based on the Wilcoxon rank-sum test for ordered categories.

5.1.4.2 Analysis Population

The ITT population, which consists of all randomized patients according to their treatment assignment in the study, was the primary efficacy analysis population.

5.1.4.3 Analysis of Primary Efficacy Data

The primary efficacy endpoint was the number of sickle cell crises through Week 48 and prior to start of taper. The definition and methods of classification of an event as an SCC are summarized in Table 14. A sickle cell crisis was defined as a visit to an emergency department or medical facility for SCD-related pain that was treated with a parenterally-administered narcotic or parenterally-administered ketorolac. Administration of oral narcotic, oral ketorolac or other oral non-narcotic pain relievers was adjudicated as a crisis only if the non-use of parental narcotic or parenteral ketorolac was clearly documented as facility policy. In addition, the occurrence of ACS (a new infiltrate on chest x-ray associated with one or more new symptoms: fever, cough, sputum production, dyspnea, or hypoxia), priapism, and splenic sequestration were considered sickle cell crises even if the symptoms were not painful enough to require narcotics or 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 grams/dL within a 24-hour period.

Sickle cell crises were recorded on the adverse event case report forms. An independent central adjudication committee (CAC) was used to evaluate whether reported sickle cell crises, as well as hospitalizations and emergency room/medical visits related to sickle cell crises, met the criteria of the efficacy outcome. The committee was composed of three physician members (hematologists and oncologists) that followed procedures detailed in a CAC Manual. Sickle cell crises that were determined by the committee to have met the relevant predefined criteria were included in the efficacy analyses, while all investigator-reported adverse events, including those adjudicated as SCCs, were included in the safety summaries.

Table 14. Parameters for Definition and Methods of Classification of an Event as an SCC

	Study 09-01
Definition of an SCC	
Visits to a medical facility	
Reason for visit	SCD-related pain
Treatment included	Parenterally administered narcotic or toradol (ketorolac)[a]
ACS[b]	Acute clinical pulmonary findings corroborated by findings of a new pulmonary infiltrate on chest x-ray films
Priapism[b]	Considered an SCC
Splenic sequestration[b]	Increase in spleen size associated with localized pain along with a ≥ 2 g/dL decrease in Hgb concentration within 24 hours
Method of classification	Central adjudication committee

ACS = acute chest syndrome, Hgb = hemoglobin, SCC = sickle cell crisis, SCD = sickle cell disease.

[a] Unless the medical facility only used non-narcotics or orally administered narcotics, or if non-narcotic pain relievers or oral narcotics were administered during the visit, the non-use of parenteral narcotic or parenteral toradol (ketorolac) was clearly documented.

[b] The events were considered to be SCCs even if the symptoms were not painful enough to require narcotics or toradol (ketorolac).

5.1.4.3.1 Methods of Imputation

Patients that discontinued had their number of crises imputed either as the mean for completed patients of the same treatment group or as the LOCF (ie, number of crises at the time of discontinuation), whichever was larger (Table 15). Additionally, sensitivity analyses of the primary parameter were performed using other methods (LOCF imputation and a time-adjusted LOCF approach that extrapolated the number of SCCs per 48 weeks).

Table 15. Methods of Imputation - Study 09-01

Method	Noncompleters	Imputed Data[a]
Used in individual CSR and in ISE		
Study 09-01 CSR specified imputation	All	Mean number of crises to the nearest integer for completed patients of the same treatment group or the number of crises at the time of early discontinuation (whichever was larger)
Used in ISE as sensitivity analyses of the primary parameter		
LOCF	All	The number of crises at the time of early discontinuation
Time-adjusted LOCF	All	The number of crises at the time of early discontinuation divided by the number of days on study medication multiplied by 336, which gave an extrapolated number of crises per 48 weeks

CSR = clinical study report, ISE = integrated summary of efficacy, LOCF = last observation carried forward.
[a] Imputed values were rounded up to the nearest whole integer.

5.1.4.3.2 Primary Statistical Analyses

An overview of the statistical analysis for Study 09-01, is provided in Appendix 1. The primary efficacy analysis population is the ITT population which consists of all randomized patients according to their treatment assignment in the study. Patients were stratified by HU usage and by geographic region. For the primary statistical analysis method, the Cochran–Mantel–Haenszel test with modified ridit application was chosen. The test of the null hypothesis of the primary endpoint in the final analysis was a two-sided comparison at an overall alpha level of 0.045 (reduced from 0.050 due to an interim analysis). The CMH analysis is a test of the difference in the distribution of events between arms. In addition, median data are provided descriptively as a measure of the treatment effect. Other analyses, including NBR and KM were also used to provide treatment effect measures as rate and hazard ratios.

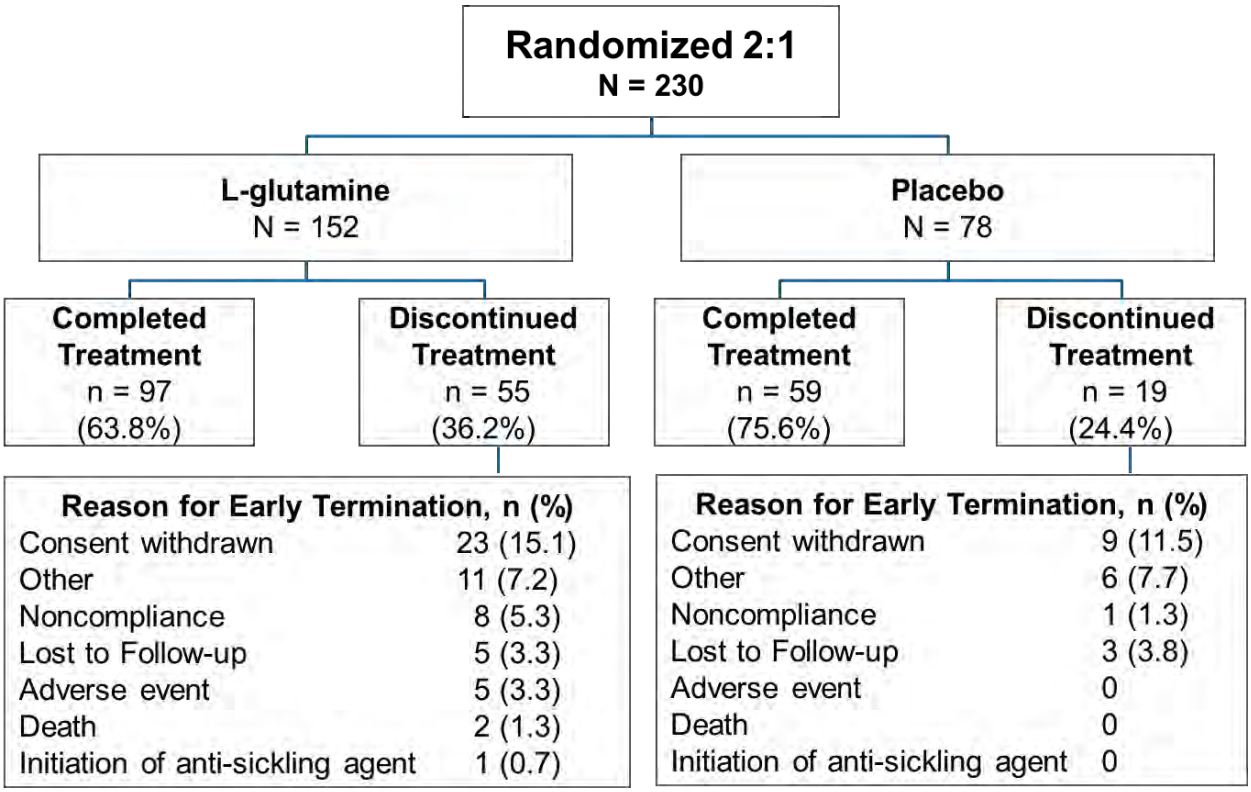
5.2 Study 09-01 Results

5.2.1 Analysis Population and Patient Disposition

Efficacy was primarily assessed in the ITT population. In Study 09-01, the ITT population consisted of all patients randomized in the study. The study used a 2:1 randomization scheme and a total of 152 and 78 patients were randomized in the L-glutamine and placebo groups, respectively (Figure 5). A total of 229 patients received at least 1 dose of study medication and were summarized for safety.

A total of 156 patients completed the study, 63.8% (97/152) in the L-glutamine group compared to 75.6% (59/78) in the placebo group (Figure 5). While there was a more than 10 point difference in the percent of patients terminating the trial early (36.2% in the L-glutamine group and 24.4% in the placebo group) sensitivity analyses demonstrated that the primary results of the trial are robust against this difference in early terminations. Reasons for discontinuation were similar between the L-glutamine and placebo groups, with the most frequent being "consent withdrawn" (15.1% and 11.5% in the L-glutamine and placebo groups, respectively), and "other" (7.2% and 7.7%). Items in the categories "consent withdrawn" and "other" included many logistical reasons, i.e. patient relocation and bone marrow transplant.

Figure 5. Study 09-01 – Patient Disposition



5.2.2 Study Population

The sample of patients who participated in this study was typical of the SCD population in the US. Sickle cell anemia was the predominate diagnosis and was similar in both treatment groups (89.5% and 91.0% in the L-glutamine and placebo groups, respectively; Table 16). After enrollment, 2 patients in the L-glutamine group were found to have Sickle ß$^{+}$ thalassemia and were withdrawn from the study but were included in the analysis. Per inclusion criteria, patients were to have had at least 2 episodes of painful crises within 12 months of screening. 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.

Prior HU use was a stratification factor, thus the percentages of patients in the L-glutamine and placebo treatment groups that had prior treatment with HU were similar (66.4% and 66.7%, respectively). The mean time since first treatment with HU was identical between treatment groups (4.4 years).

Table 16. Disease and Treatment History - Study 09-01

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

Hgb SC = sickle cell trait, HU = hydroxyurea, SCCs = sickle cell crises, SD = standard deviation.
a. Primary efficacy population in Study 09-01 (ITT).

The 2 groups of patients were well matched with respect to age, and ethnicity (Table 17). The mean age was 22.4 and 21.4 years in the L-glutamine and placebo groups, respectively, with an overall range from 5 to 58 years. A total of 49.3% of the patients in the L-glutamine group and 55.1% of the patients in the placebo group were 18 years old or younger. The majority of

patients were Black (94.7% and 93.6% in the L-glutamine and placebo groups, respectively) and had a diagnosis of sickle cell anemia (approximately 90% in both groups). The majority of patients were female in both groups (approximately 52% in the L-glutamine group and about 58% in the placebo group).

Table 17. Demographic Characteristics – Study 09-01

	L-glutamine N = 152	Placebo N = 78
Age (years)		
Mean (SD)	22.4 (12.32)	21.4 (12.42)
Range	5 - 57	5 - 58
Groups, n (%)		
≤ 18 years	75 (49.3)	43 (55.1)
> 18 years	77 (50.7)	35 (44.9)
Sex, n (%)		
Male	73 (48.0)	33 (42.3)
Female	79 (52.0)	45 (57.7)
Race, n (%)		
Black	144 (94.7)	73 (93.6)
Hispanic	4 (2.6)	3 (3.8)
Caucasian	--	--
Asian	--	--
Other	4 (2.6)	2 (2.6)

SD = standard deviation.

The median number of days on study was similar in the L-glutamine group and the placebo group (Table 18). The percentage of study medication taken was the same in the 2 groups.

Table 18. Study Medication Dosing - Study 09-01

	All Randomized[a]	
	L-glutamine N = 152	Placebo N = 78
Number of Days on Study		
N	152	78
Mean (SD)	293.6 (123.15)	327.4 (103.52)
Median	368	372
Range	2-449	29 - 442
% of Study Medication Taken		
N	137	75
Mean (SD)	72.0 (22.04)	72.8 (23.90)
Median	77.4	76.6
Range	0-117.2	0-102.1

SD = standard deviation.

5.2.3 CMH Analyses of the Number of SCCs

The result of the primary analysis of the number of SCCs in the ITT population using the CMH test with modified ridit scores and the prespecified imputation rules is presented in Table 19. The results of the sensitivity analyses of the primary endpoint run using CMH tests are also summarized in the same table.

The results of the primary analysis demonstrated statistically significantly fewer ($p = 0.0052$) SCCs in favor of the L-glutamine treatment group relative to placebo. The median number of SCCs in the L-glutamine treatment group was 25% less or 1 SCC lower than for placebo. The sensitivity analyses intended to test the effect of imputation methods (LOCF and time-adjusted LOCF) demonstrated statistically significantly fewer SCCs in favor of the L-glutamine treatment group ($p = 0.0025$ and $p = 0.0190$, respectively). Median numbers of SCCs were approximately 33% and 50% or 1 and 2 SCCs lower than placebo, respectively. The results of all other sensitivity analyses were similar to the results for the primary endpoint (ie, statistically significant in favor of L-glutamine).

Table 19. Primary Analysis: CMH Analyses of the Number of SCCs - Study 09-01

	L-glutamine N = 152	Placebo N = 78
Primary analysis[a] Number of SCCs using modified ridit scores and CSR imputation rules:		
P-value (controlling for region and HU use)	0.0052	
Descriptive Statistics		
Mean (SD)	3.2 (2.24)	3.9 (2.54)
Median (min, max)	3.0 (0, 15)	4.0 (0, 15)
Frequency Distribution of Sickle Cell Crisis (%)[b]		
0	15 (10)	4 (5)
1	16 (11)	10 (13)
2	17 (11)	11 (14)
3	62 (41)	4 (5)
4	16 (11)	23 (29)
5	8 (5)	12(15)
6	6 (4)	5 (6)
7	5 (3)	4 (5)
8	2 (1)	2 (3)
9	3 (2)	1 (1)
11	1 (1)	1 (1)
15	1 (1)	1 (1)
Sensitivity analyses		
Number of SCCs using modified ridit scores with other stratification factors and CSR imputation rules		
P-value (controlling for HU use but not region)	0.0041	
P-value (controlling for region but not HU use)	0.0067	
P-value (controlling for neither region nor HU use)	0.0039	
Number of SCCs ranked prior to analysis and using CSR imputation rules		
P-value (controlling for region and HU use)	0.0052	
Number of SCCs using modified ridit scores and LOCF		
P-value (controlling for region and HU use)	0.0025	
Descriptive Statistics		
Mean (SD)	2.5 (2.56)	3.5 (2.74)
Median (min, max)	2.0 (0, 15)	3.0 (0, 15)
Number of SCCs using modified ridit scores and time-adjusted LOCF		
P-value (controlling for region and HU use)	0.0190	
Descriptive Statistics		
Mean (SD)	3.6 (4.34)	6.8 (19.09)
Median (min, max)	2.0 (0, 28)	4.0 (0, 168)

CMH = Cochran-ManteL-Haenszel, CSR = clinical study report, HU = hydroxyurea, LOCF = last observation carried forward, SCCs = sickle cell crises, SD = standard deviation.

[a] CSR specified endpoint analyzed by CMH using modified ridit scores – ITT population.

[b] CSR imputation rules

5.2.3.1 Effect of Imputation on the Primary Analysis

The discontinuation rate in Study 09-01was 36.2% for the L-glutamine group and 24.4% for the placebo group (Section 5.2.1). The imputation method used in the primary analysis may have favored the placebo group, as explained below. For patients who discontinued prior to Week 48, painful SCC count was imputed using the mean number of crises for the patients of the same treatment group who did complete Week 48. If the imputed count was less than the crises count at the time of discontinuation, the latter was used (Section 5.1.4.3.1).

The mean number of sickle cell crises (SCCs) for the study completers (rounded to nearest integer) was 4 for the placebo group and 3 for the L-glutamine group. Thus, for patients who discontinued early, SCC counts less than 4 for the placebo group would be imputed to 4 and SCC counts less than 3 for the L-glutamine would be imputed to 3. Crisis counts equal to or higher than the mean of the respective treatment group would not be imputed.

The number of patients with SCC counts that would be imputed is given in Table 20.

Table 20. Number of Patients With Imputed SCC Counts - Study 09-01

	Number of Patients With Imputed SCC Counts	
	L-glutamine	Placebo
Number of SCCs at Discontinuation		
0	20	4
1	20	2
2	6	4
3	0	4

SCC = sickle cell crisis.

Of the 152 patients in the L-glutamine group, 46 patients (30.3%) had SCC counts of 0, 1 or 2 that were then imputed to 3, while of the 78 patients in the placebo group, 14 (17.9%) had SCC counts of 0, 1, 2, or 3 that were then imputed to 4. Since a larger percentage of L-glutamine patients had low numbers of SCCs that were imputed to a higher number, it is unlikely that the imputation method resulted in an advantage for L-glutamine in the analysis. It is likely that instead the imputation favored the placebo group.

5.2.4 NBR Analysis of the Rate of SCCs per 48 Weeks

Another sensitivity analysis was performed using NBR. An NBR model with the log of time in study as an offset allows the data from patients who withdrew before Week 48 to be used without imputation. The model takes time on study into account by transforming the number of events into rates. The results of the NBR analysis (observed data; no imputation) of the rate of SCCs per 48 weeks are presented in Table 21.

This analysis demonstrated a lower rate of SCCs in the L-glutamine treatment group relative to the placebo group ($p = 0.0374$) with a rate ratio (0.78) in favor of patients that received L-glutamine.

Table 21. Rate of SCCs Per 48 Weeks - Study 09-01

	Study 09-01	
NBR Modeling Results	**L-glutamine N = 152**	**Placebo N = 78**
P-value	0.0374	
Rate per 48 weeks (95% CI)	3.25 (2.76, 3.83)	4.19 (3.44, 5.11)
Rate ratio[a] (95% CI)	0.78 (0.61, 0.99)	

CI = confidence interval, NBR = negative regression, SCCs = sickle cell crises.
[a] Rate ratio is (rate per 48 weeks for L-glutamine)/(rate per 48 weeks for placebo). A rate ratio < 1 favors L-glutamine.

5.2.5 Additional Efficacy Endpoints

5.2.5.1 Time to First and Second SCC

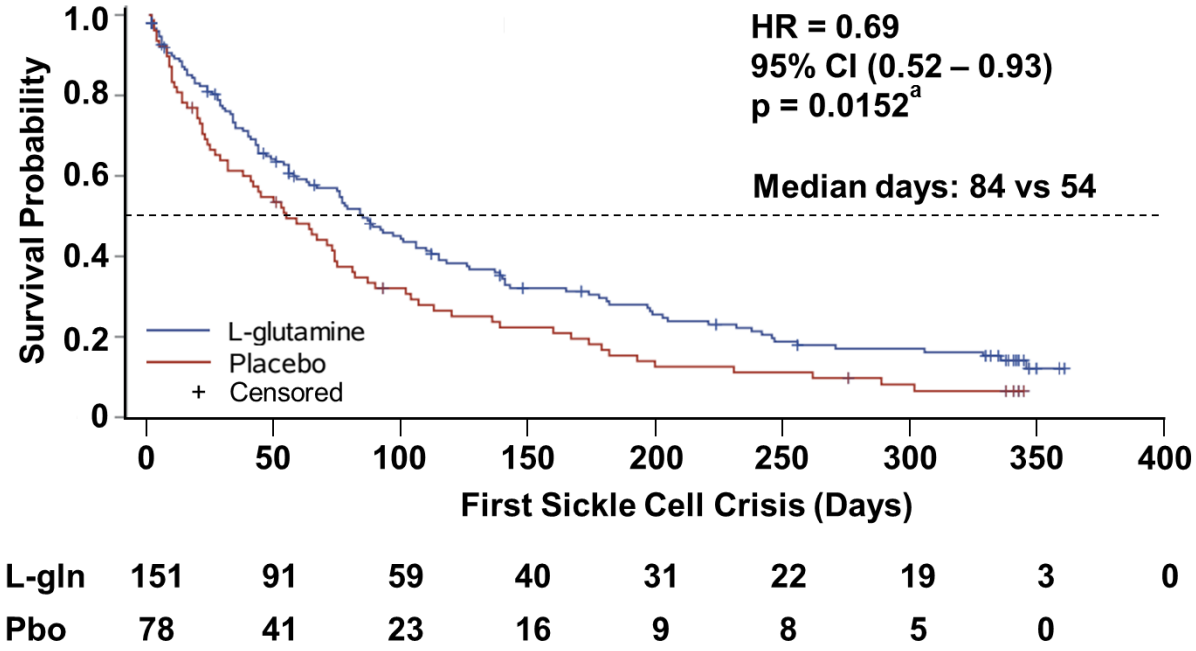
A time to first SCC analysis was conducted per the ISE SAP and a time to second SCC analysis was subsequently conduced as a *post hoc* analysis. These analyses are not sensitive to early terminations and provide a between-arm comparison of the rate of SCC events through the hazard ratio estimate. These data were analyzed for statistical significance via Log-Rank test (Table 22). For time to first SCC, there was a significant difference between the treatment groups (p = 0.0152). The crisis-free survival probability remained greater in the L-glutamine treatment group relative to the placebo group throughout the duration of the study (Figure 6). At the 50th percentile level, time to first crisis was 84 days in the L-glutamine vs 54 days in the placebo group, a difference of 30 days. The hazard ratio for this analysis was 0.69. This result shows that the primary analysis is robust against the between-arm imbalance in early terminations.

Table 22. Time to First SCC Log Rank Test - Study 09-01

Point Estimate (95% CI) of the Quartiles of the Survival Curve (Days)	**Study 09-01**	
	L-glutamine	**Placebo**
P-value[a]	0.0152	
75th	202.0 (142.0, 270.0)	135.0 (81.0, 181.0)
50th (Median)	84.0 (62.0, 109.0)	54.0 (31.0, 73.0)
25th	33.0 (23.0, 42.0)	19.0 (9.0, 26.0)

Censoring date is the earlier of the date of taper period start and the study exit date.
CI = confidence interval, SCC = sickle cell crisis.
[a] Log Rank Test

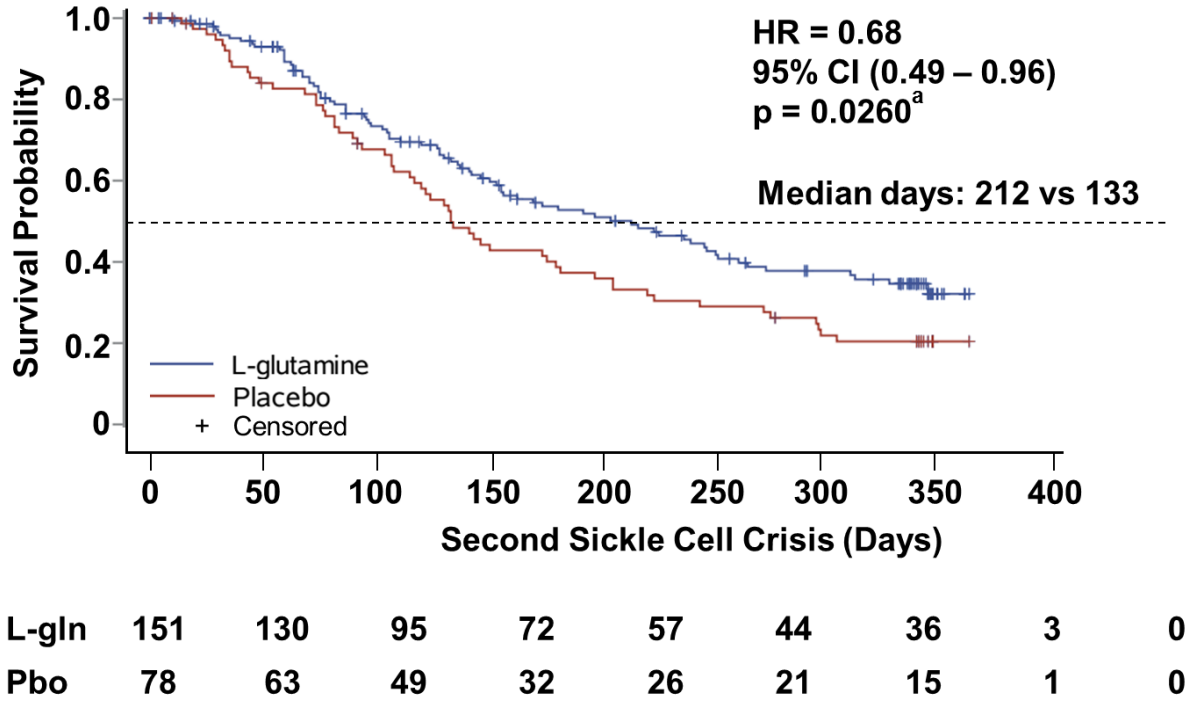
Figure 6. Time to First Crisis: Study 09-01



[a] Log-Rank test.
CI = confidence interval, HR = hazard ratio, L-gln = L-glutamine, Pbo = placebo.

For time to second SCC, there was also significant difference between the treatment groups (p = 0.0260). At the 50th percentile level, time second crisis (measured from the beginning of the study) was 212 days in the L-glutamine vs 133 days in the placebo group, a difference of 79 days. The hazard ratio for this analysis was 0.68 (Figure 7).

Figure 7. Time to Second Crisis: Study 09-01



[a] Log-Rank test.
CI = confidence interval, HR = hazard ratio, L-gln = L-glutamine, Pbo = placebo.

5.2.5.2 Occurrences of Acute Chest Syndrome

There was a statistically significant difference (p = 0.0028) in the number of occurrences of ACS. The mean number of ACS occurrences was approximately 67% or 0.2 fewer for the L-glutamine group than for placebo (Table 23).

Table 23. CMH Analyses of Number of Occurrences of ACS Using Modified Ridit Scores

	Study 09-01	
Number of occurrences of ACSa	**L-glutamine N = 152**	**Placebo N = 78**
P-value (controlling for region and HU use)	0.0028	
Descriptive statistics		
Mean (SD)	0.1 (0.37)	0.3 (0.63)
Median (min, max)	0 (0, 2)	0 (0, 3)
Number of ACS Occurrences n (%)		
0	139 (91)	60 (77)
1	10 (7)	13 (17)
2	3 (2)	4 (5)
3	0	1 (1)

ACS = acute chest syndrome, CMH = Cochran-Mantel-Haenszel, HU = hydroxyurea, SD = standard deviation.

5.2.5.3 Hospitalizations and ER Visits for Sickle Cell Pain

The number of hospitalizations for sickle cell pain and ER visits for sickle cell pain are presented in Table 24. The median number of hospitalizations for sickle cell pain was approximately 33% lower or 1 hospitalization fewer for the L-glutamine group than for placebo. The difference in the number of hospitalizations for sickle cell pain was statistically significant (p = 0.0045) and favored the L-glutamine group. The median number of ER visits for sickle cell pain was the same across treatment groups.

Table 24. CMH Analyses of Number of Hospitalizations or ER Visits for Sickle Cell Pain Using Modified Ridit Scores

	Study 09-01	
Endpoints	**L-glutamine N = 152**	**Placebo N = 78**
Number of hospitalizations for sickle cell pain		
P-value (controlling for region and HU use)	0.0045	
Descriptive statistics		
Mean (SD)	2.3 (1.99)	3.0 (2.33)
Median (min, max)	2.0 (0, 14)	3.0 (0, 13)
Number of ER visits		
P-value (controlling for region and HU use)	0.0888	
Descriptive statistics		
Mean (SD)	1.1 (1.49)	1.5 (2.29)
Median (min, max)	1.0 (0, 12)	1.0 (0, 15)

CMH = Cochran-Mantel-Haenszel, ER = emergency visits, HU = hydroxyurea, SD = standard deviation.

5.2.5.4 Percentage of Time Hospitalized

The percentage of time hospitalized was analyzed for the ITT population using an analysis of variance (ANOVA) model with treatment as the main effect (Table 25). The median percentage of time hospitalized was 2.2% for the L-glutamine treatment group and 3.6% in the placebo group.

Table 25. Percentage of Time Hospitalized

	Study 09-01	
Percentage of Time Hospitalized[a]	**L-glutamine N = 152**	**Placebo N = 78**
P-value	0.1794	
Descriptive statistics		
Mean (SD)	4.7 (6.51)	6.0 (8.44)
Median (min, max)	2.2 (0, 38)	3.6 (0, 56)
LS mean (SE)	4.7 (0.585)	6.0 (0.817)
95% CI	3.513, 5.820	4.410, 7.631
LS mean difference (SE)[b]	-1.354 (1.005)	
95% CI	-3.335, 0.627	

CI = confidence interval, LS = least squares, SD = standard deviation, SE = standard error.

[a] The percentage of time hospitalized is the cumulative duration of hospitalization divided by the length of time on study times 100.

[b] Difference is L-glutamine minus placebo.

5.2.5.5 Cumulative Days in Hospital

As reported in the clinical study report (CSR) an analysis for the cumulative days in hospital through Week 48 was performed. The median number of days in hospital was statistically significantly shorter in the L-glutamine group (6.5 days) compared to 11 days in the placebo group (Table 26). The difference between the groups in days hospitalized was statistically significant using the Wilcoxon rank-sum test (p=0.022).

Table 26. Cumulative Days in Hospital

	Study 09-01	
Cumulative Days Hospitalized	**L-glutamine N = 152**	**Placebo N = 78**
P-value	p=0.022[a]	
Descriptive statistics		
Mean (SD)	12.1 (16.6)	18.1 (27.4)
Median	6.5	11
Range	0–94	0–187

SD = standard deviation. Range = min-max

[a] P-values are from Wilcoxon rank-sum test

5.2.5.6 Hematologic Parameters

No major shifts from baseline were observed for changes in hematocrit, hemoglobin, and reticulocyte counts at Weeks 4, 8, 12, 16, 20, 24, 32, 40, and 48.

5.2.5.7 Blood Transfusions

Patients in the L-glutamine group had fewer episodes of transfusion during the study (Table 27). In the L-glutamine group, 47.4% of patients had at least one simple transfusion

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

Table 27. Summary of Blood Exchange Transfusions and Simple Transfusions

	Study 09-01	
	L-glutamine N = 152	**Placebo N = 78**
	N (%)	N (%)
Blood Exchange Transfusions[a]		
Patients with at Least One Transfusion	3 (2.0)	5 (6.4)
Number of Transfusions		
1	2 (1.3)	5 (6.4)
2	1 (0.7)	0
3	0	0
> 3	0	0
Total number of Exchange Transfusions	4	5
Simple Transfusions[a]		
Patients with at Least One Transfusion	72 (47.4)	40 (51.3)
Number of Transfusions		
1	25 (16.4)	10 (12.8)
2	22 (14.5)	7 (9.0)
3	6 (3.9)	4 (5.1)
> 3	19 (12.5)	19 (24.4)
Total number of Simple Transfusions	216	181
Exchange or Simple Transfusion		
Patients with at Least One Transfusion[b]	74 (48.7)	42 (53.8)
Number of Transfusions		
1	26 (17.1)	12 (15.4)
2	23 (15.1)	7 (9.0)
3	6 (3.9)	3 (3.8)
> 3	19 (12.5)	20 (25.7)
Total number of Exchange and Simple Transfusions	220	186

[a] Transfusion information is based on data recorded on the Blood Products case report form. Other blood products such as fresh frozen plasma and platelets are not included.

[b] 4 patients (L-glutamine: 02-516; placebo: 02-501, 02-508, and 18-504) in Study 09-01 had both, Blood Exchange Transfusion and Simple Transfusion; we are considering patients only once.

5.2.6 Subgroup Analyses

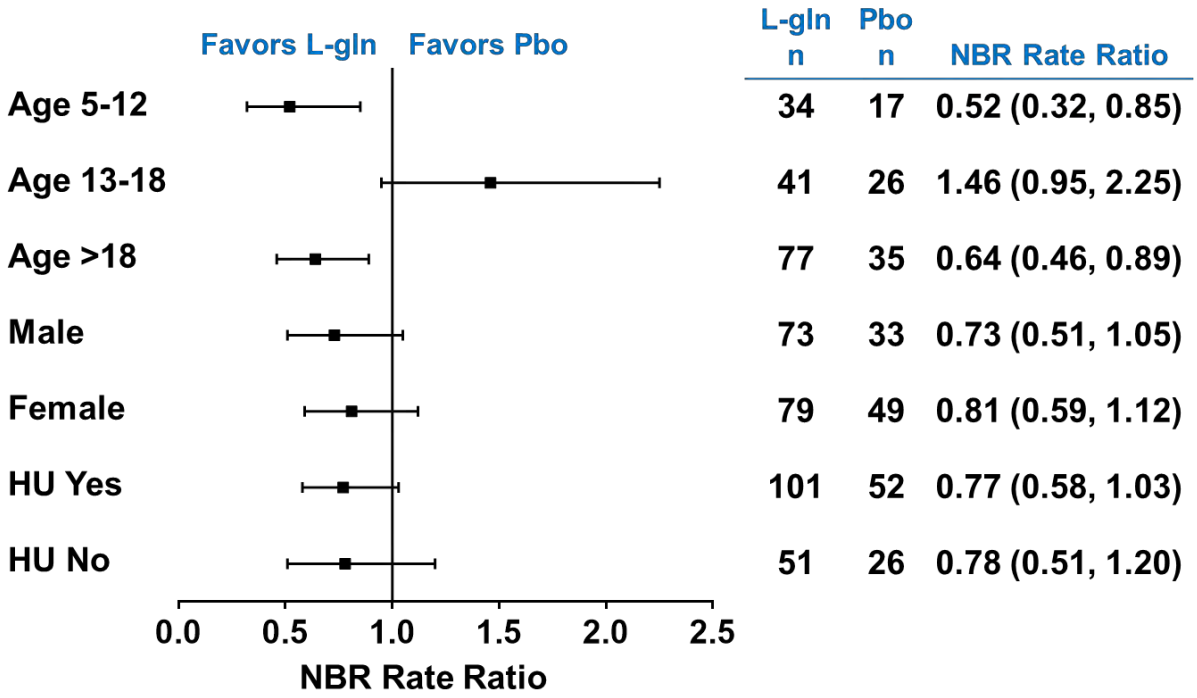
Subgroup analyses were performed for the primary efficacy endpoint to determine if the treatment effect varied by subgroup in Study 09-01. The results of these analyses were expressed in terms of the number of SCCs at 48 weeks. An NBR model was utilized, as it provided a rate ratio, which is a readily interpretable estimate of treatment effect. The model included log (time on study) as an offset variable, treatment and subgroup main effects, and a treatment by subgroup interaction term. The NBR analysis is specifically intended for count data and does not require imputation. For each subgroup, the event rate for each treatment group and its 95% CI, and the rate ratio (L-glutamine: placebo) and its 95% CI were calculated. The p-values for subgroup effect and treatment by subgroup interaction were reported.

In this analysis, age did not have a statistically significant effect ($p = 0.2403$), however there was a statistically significant treatment by age interaction ($p = 0.0026$) (Table 28). Patients in the 5 - 12 years subgroup had a lower rate of SCCs per 48 weeks in the L-glutamine treatment group (2.54) relative to placebo (4.85) and a rate ratio in favor of L-glutamine (0.52) (Figure 8). Patients in the 13 – 18 years subgroup had a higher rate of SCCs per 48 weeks in the L-glutamine treatment group (3.95) relative to placebo (2.70) and a rate ratio in favor of placebo (1.46). Patients in the > 18 years subgroup demonstrated a lower rate of SCCs in the L-glutamine treatment group (3.28) relative to placebo (5.14) and a rate ratio in favor of L-glutamine (0.64).

In the sex subgroups, sex had no statistically significant effect on the overall rate of SCCs ($p = 0.9281$). There was also no statistically significant treatment by sex interaction ($p = 0.6830$). Patients in the male subgroup demonstrated a lower rate of SCCs in the L-glutamine treatment group (3.15) relative to placebo (4.29), with a rate ratio in favor of L-glutamine (0.73). Similarly, patients in the female subgroup demonstrated a lower rate of SCCs in the L-glutamine treatment group (3.35) relative to placebo (4.12) and a rate ratio in favor of L-glutamine (0.81).

In the baseline HU use subgroups, HU use did not demonstrate a statistically significant effect on the overall rate of SCCs ($p = 0.4429$), nor was there a statistically significant treatment by HU use interaction ($p = 0.9612$). Patients that were taking HU at baseline demonstrated lower rates of SCCs in the L-glutamine treatment group (3.41) relative to placebo (4.42), with a rate ratio in favor of L-glutamine (0.77). Patients that were not taking HU at baseline also demonstrated lower rates of SCCs in the L-glutamine treatment group (3.10) relative to placebo (3.97), with a rate ratio in favor of L-glutamine (0.78).

Figure 8. NBR Subgroup Analysis Rate Ratios by Age, Sex, and Hydroxyurea use – Study 09-01



NBR = negative binomial regression, HU = hydroxyurea.

Table 28. NBR Analysis of Number of SCCs at Week 48 by Age, Sex, and Hydroxyurea use Study 09-01

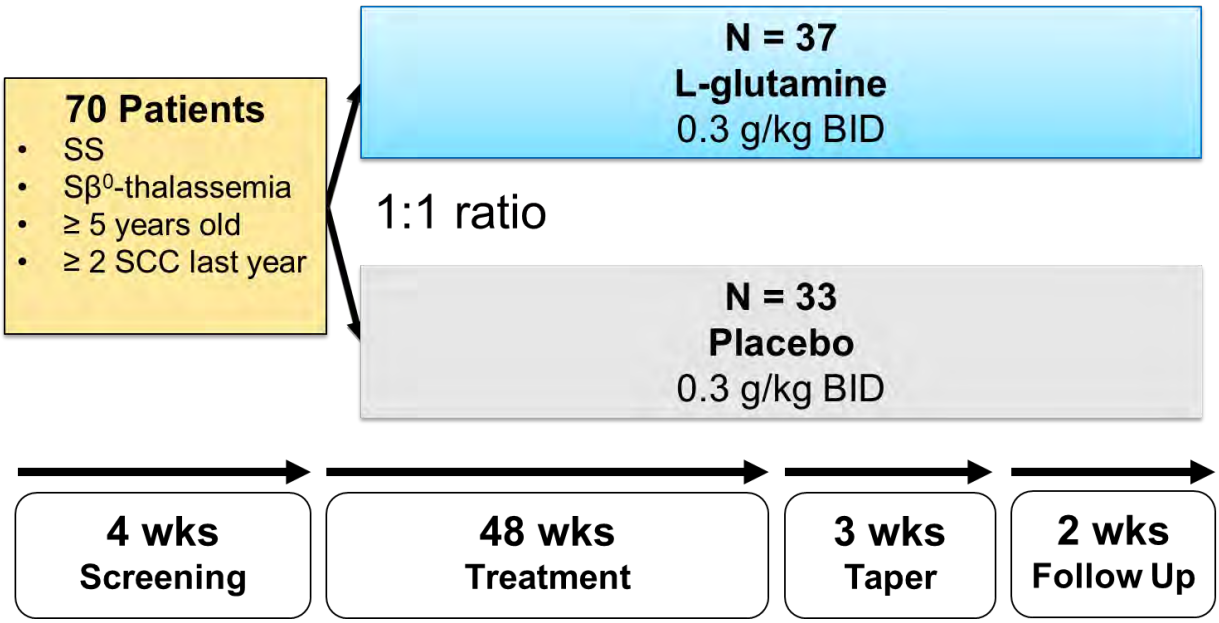
Subgroup Statistics	Placebo N = 78	L-glutamine N = 152
5 - 12 years		
Number of Patients	17	34
Rate Per 48 Weeks (95% CI)	4.85 (3.30, 7.13)	2.54 (1.85, 3.47)
Rate Ratio[a] (95% CI)	0.52 (0.32, 0.85)	
13 - 18 years		
Number of Patients	26	41
Rate Per 48 Weeks (95% CI)	2.70 (1.92, 3.79)	3.95 (2.97, 5.25)
Rate Ratio[a] (95% CI)	1.46 (0.95, 2.25)	
> 18 years		
Number of Patients	35	76
Rate Per 48 Weeks (95% CI)	5.14 (3.92, 6.75)	3.28 (2.65, 4.04)
Rate Ratio[a] (95% CI)	0.64 (0.46, 0.89)	
P-value for H_0: no treatment by age interaction	0.0026	
P-value for H_0: no age effect	0.2403	
Sex: Male		
Number of Patients	33	72
Rate Per 48 Weeks (95% CI)	4.29 (3.18, 5.78)	3.15 (2.51, 3.94)
Rate Ratio[a] (95% CI)	0.73 (0.51, 1.05)	
Sex: Female		
Number of Patients	45	79
Rate Per 48 Weeks (95% CI)	4.12 (3.19, 5.33)	3.35 (2.70, 4.14)
Rate Ratio[a] (95% CI)	0.81 (0.59, 1.12)	
P-value for H_0: no treatment by sex interaction	0.6830	
P-value for H_0: no sex effect	0.9281	
Hydroxyurea use at baseline: Yes		
Number of Patients	52	101
Rate Per 48 Weeks (95% CI)	4.42 (3.50, 5.59)	3.41 (2.85, 4.09)
Rate Ratio[a] (95% CI)	0.77 (0.58, 1.03)	
Hydroxyurea use at baseline: No		
Number of Patients	26	50
Rate Per 48 Weeks (95% CI)	3.97 (2.86, 5.50)	3.10 (2.34, 4.11)
Rate Ratio[a] (95% CI)	0.78 (0.51, 1.20)	
P-value for H_0: no treatment by HU use interaction	0.9612	
P-value for H_0: no HU use effect	0.4429	

NBR model with Treatment, Sex, Region and Hydroxyurea Use as main effects, a Treatment by Sex interaction term, and log (time on study) as an offset.
One patient who was randomized but never took study medication is not included in this analysis.
CI = confidence interval, H_0 = null hypothesis, NBR = negative binomial regression, SCCs = sickle cell crises.
[a] Rate ratio is (rate per 48 weeks for L-glutamine)/(rate per 48 weeks for placebo). A rate ratio < 1 favors L-glutamine.

5.3 Supportive Data from Phase 2 Study 10478

The designs of Study 10478 and Study 09-01 were very similar. Study 10478 was a Phase 2 randomized, double-blind, placebo-controlled, parallel-group, multicenter study that enrolled patients who were at least 5 years of age with sickle cell anemia or sickle β^0-thalassemia. Patients were to have had ≥ 2 episodes of painful crises within the 12 months prior to the screening visit. As with 09-01, the study consisted of a 4-week screening period, a 48-week treatment period, a 3-week tapering period, and a 2-week follow-up period for a total duration of up to 57 weeks. Study 10478 differed in that patient were randomized in a 1:1 ratio.

Figure 9. Study Design – Study 10478



BID = twice a day, SCC = sickle cell crisis, SS = sickle cell disease.

Additional differences between the studies are summarized below:

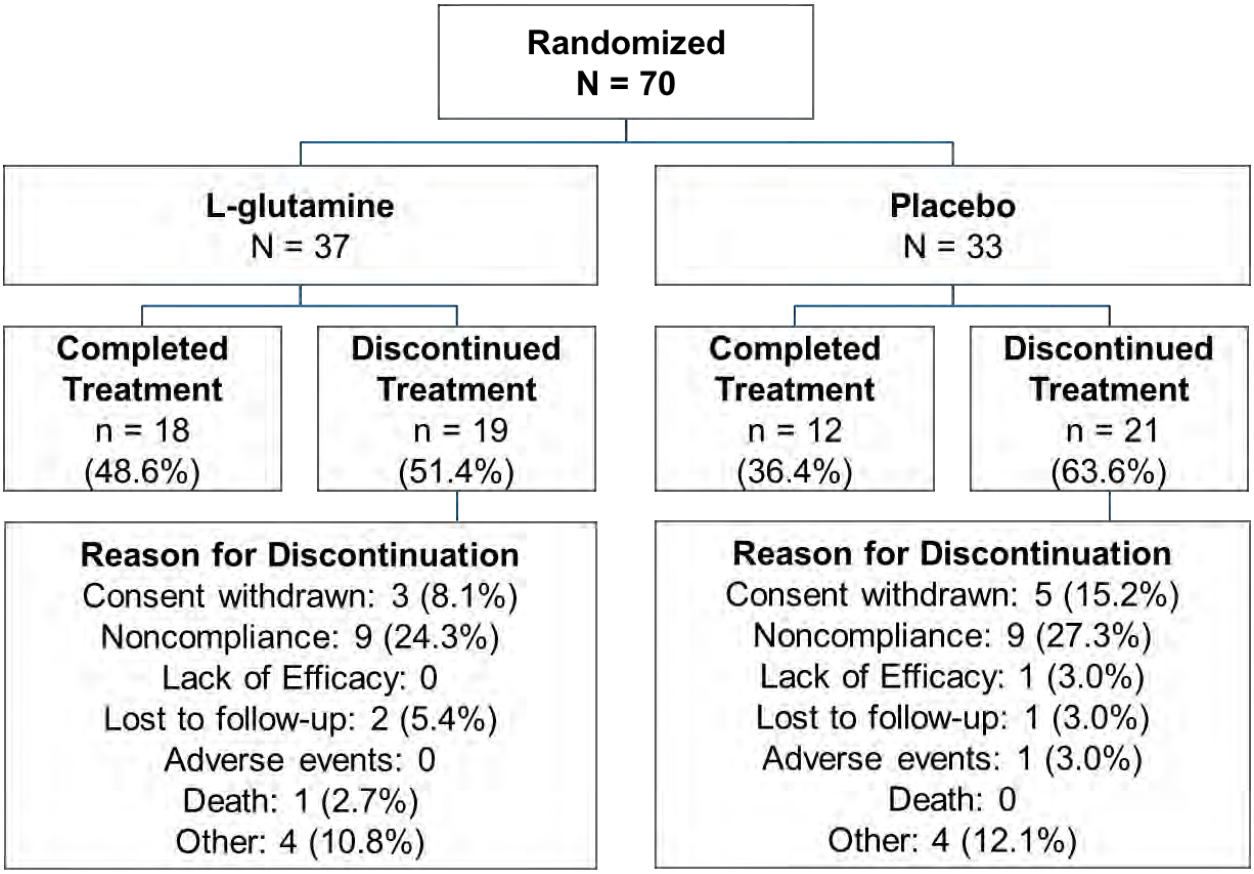
- Although both studies required a visit to a medical facility, Study 10478 required that the medical facility visit last 4 hours or longer, while Study 09-01 did not require a minimum time duration.
- In Study 10478, the occurrence of hepatic (liver) sequestration was considered a crisis, while, in Study 09-01 it was not.
- In Study 10478, a programming algorithm was used to determine whether or not an event was considered a crisis, while in Study 09-01, the decision was made by a central adjudication committee.
- In Study 10478, events determined to be crises were not required to be separated by ≥ 24 hours; however, in Study 09-01 events adjudicated as crises were separated by

≥ 24 hours (i.e., the difference between the end date of 1 crisis and the start date of the next crisis was required to be at least 2 calendar days).

5.3.1 Analysis Population and Patient Disposition

Efficacy was primarily assessed in the ITT population. In Study 10478, the ITT population consisted of all 70 patients randomized in the study. A total of 37 and 33 patients were randomized in the L-glutamine and placebo groups, respectively. Thirty patients completed the study, 48.6% (18/37) in the L-glutamine group and 36.4% (12/33) in the placebo group. Reasons for early termination were similar between the groups, with the most frequent being noncompliance (24.3% and 27.3% in the L-glutamine and placebo groups, respectively), consent withdrawn (8.1% and 15.2%), and "other" (10.8% and 12.1%). Subjects from one site were not included in the analysis due to potential investigator misconduct and suspected fraud. The suspected fraud was discovered by Emmaus during study monitoring visits, and results from the 11 patients enrolled there (5 randomized to L-glutamine and 6 to placebo) were excluded from the primary analyses.

Figure 10. Patient Disposition-Study 10478



5.3.2 Study Population

Sickle cell anemia was the predominant diagnosis in study 10478 (89.2% and 84.8% in the L-glutamine and placebo groups, respectively). Prior HU use was not a stratification factor and varied between the treatment groups (62.2% of patients in the L-glutamine group reported prior HU use compared to 39.4% in the placebo group). In addition, patients in the L-glutamine group had been treated with HU more recently than those in the placebo group (1.3 vs 88.7 days, respectively).

The age distribution of patients was similar in the 2 treatment groups, with the majority of patients over the age of 18 (86.5% in the L-glutamine group and 84.8% in the placebo group) (Table 29). There were higher percentages of females in the L-glutamine group and males in the placebo group (L-glutamine: 32.4% [male] and 67.6% [female]; placebo: 60.6% [male] and 39.4% [female]). The majority of patients were Black in both treatment groups.

Table 29. Demographic Information – Study 10478

	L-glutamine N = 37	**Placebo N = 33**
Age (years)		
Mean (SD)	29.8 (10.66)	27.2 (10.21)
Range	11 - 58	9 - 55
Groups, n (%)		
≤ 18 years	5 (13.5)	5 (15.2)
> 18 years	32 (86.5)	28 (84.8)
Sex, n (%)		
Male	12 (32.4)	20 (60.6)
Female	25 (67.6)	13 (39.4)
Race, n (%)		
Black	36 (97.3)	32 (97.0)
Hispanic	1 (2.7)	1 (3.0)

SD = standard deviation.

5.3.3 CMH Analyses of the Number of SCCs

The result of the primary analysis of the number of SCCs in the ITT population using the CMH test with modified ridit scores and the individual CSR imputation rules is presented in Table 30. The results of the sensitivity analyses of the primary endpoint using CMH tests are also summarized in the same table.

For the primary endpoint, there was a tendency toward fewer painful sickle cell crises in the L-glutamine group compared to the placebo group but the between-group differences were not statistically significant (Table 30). Two sensitivity analyses intended to test the effect of imputation methods (LOCF and time-adjusted LOCF) showed the median number of SCCs in the L-glutamine group was 50% lower in the LOCF analysis (1 vs 2 events) and 33% lower in the time-adjusted LOCF analysis (2 vs 3 events) relative to the placebo group.

Table 30. CMH Analyses of the Number of SCCs - Study 10478

	L-glutamine N = 37	Placebo N = 33
Primary analysis[a]: CMH using modified ridit scores		
P-value (controlling for center)	0.1501	
Number of SCCs using modified ridit scores and CSR imputation rules		
Descriptive statistics		
Mean (SD)	4.3 (5.22)	9.6 (17.88)
Median (min, max)	4.0 (0, 27)	4.0 (0, 90)
Sensitivity analyses		
Number of SCCs using modified ridit scores and LOCF		
P-value (controlling for center)	0.3207	
Descriptive statistics		
Mean (SD)	3.0 (4.88)	6.5 (14.82)
Median (min, max)	1.0 (0, 27)	2.0 (0, 82)
Number of SCCs using modified ridit scores and time-adjusted LOCF		
P-value (controlling for center)	0.3611	
Descriptive statistics		
Mean (SD)	6.1 (13.22)	10.4 (19.36)
Median (min, max)	2.0 (0, 77)	3.0 (0, 90)

CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, LOCF = last observation carried forward, SCCs = sickle cell crises, SD = standard deviation.

[a] The primary analysis was re-analyzed using a CMH analysis of the number of SCCs using modified ridit scores.

5.3.4 NBR Analysis of the Rate of SCCs per 48 Weeks

The NBR model with the log of time in study as an offset allows the data from patients who withdrew before Week 48 to be used without imputation. The model takes time on study into account by transforming the number of events into rates. Negative binomial regression analysis demonstrated a statistically significantly lower rate of SCCs in the L-glutamine treatment group relative to the placebo group (p = 0.0240) with a rate ratio (0.47) in favor of patients that received L-glutamine (Table 31).

Table 31. Rate of SCCs Per 48 Weeks - Study 10478

	Study 10478	
NBR Modeling Results	**L-glutamine N = 37**	**L-glutamine N = 37**
P-value	0.0240	
Rate per 48 weeks (95% CI)	4.25 (2.47, 7.32)	9.07 (5.35, 15.38)
Rate ratio[a] (95% CI)	0.47 (0.24, 0.91)	

CI = confidence interval, NBR = negative regression, SCCs = sickle cell crises.

[a] Rate ratio is (rate per 48 weeks for L-glutamine)/(rate per 48 weeks for placebo). A rate ratio < 1 favors L-glutamine.

5.3.5 Additional Efficacy Endpoints

Results for hospitalizations and ER visits for sickle cell pain, time to first SCC, and percentage of time hospitalized are presented below. There were only 2 occurrences of ACS in Study 10478, hence an analysis was not performed for this study.

5.3.5.1 Hospitalizations and ER Visits for Sickle Cell Pain

The number of hospitalizations or ER visits is presented in Table 32. The median number of hospitalizations for sickle cell pain was 50% lower or 1 hospitalization fewer for the L-glutamine group than for placebo (1 vs 2, respectively). The median number of ER visits for sickle cell pain was the same across treatment groups.

Table 32. CMH Analyses of Number of Hospitalizations or ER Visits for Sickle Cell Pain Using Modified Ridit Scores – ITT Population (CSR Imputation Rules)

	Study 10478	
Endpoints	**L-glutamine N = 37**	**Placebo N = 33**
Number of hospitalizations for sickle cell pain		
P-value (controlling for center)	0.1104	
Descriptive statistics		
Mean (SD)	1.4 (2.34)	1.9 (2.28)
Median (min, max)	1.0 (0, 10)	2.0 (0, 10)
Number of ER visits		
P-value (controlling for center)	0.5657	
Descriptive statistics		
Mean (SD)	3.4 (5.44)	8.3 (19.03)
Median (min, max)	2.0 (0, 27)	2.0 (0, 94)

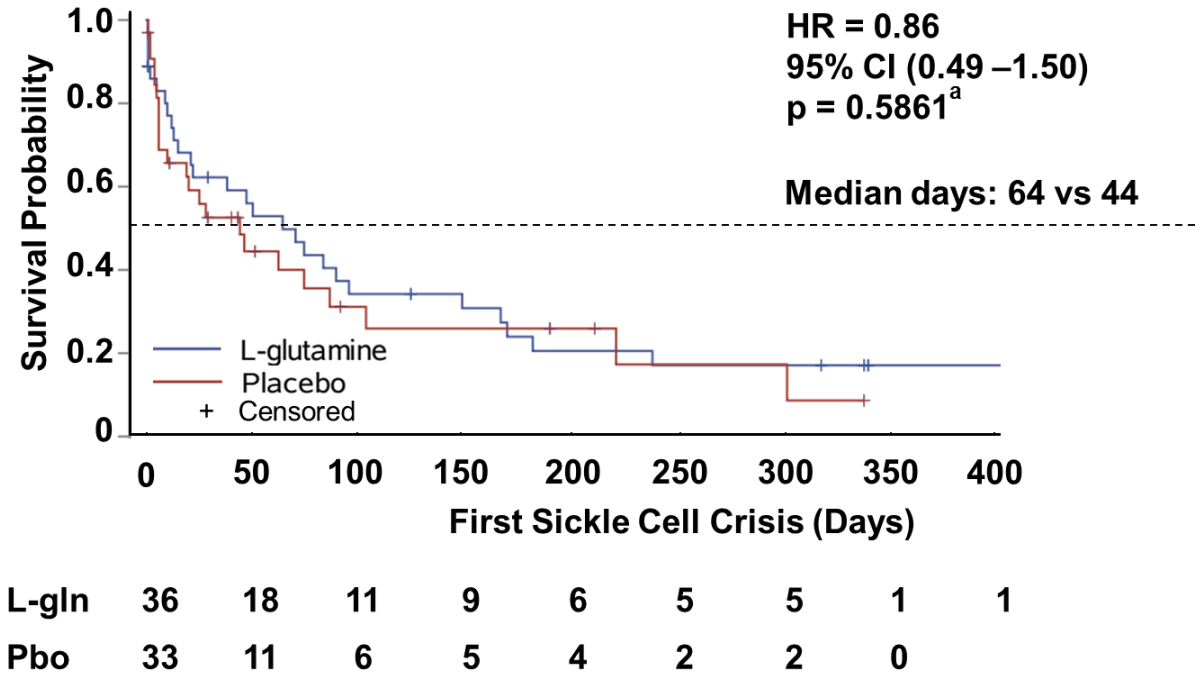
CMH = Cochran-Mantel-Haenszel, CSR = clinical study report, ER = emergency visits, SD = standard deviation.

5.3.5.2 Time to First SCC

L-glutamine delayed the onset of the first SCC in Study 10478. This is displayed graphically as crisis-free survival curves via the Kaplan-Meier method in Figure 11.

The crisis-free survival probability was greater in the L-glutamine treatment group for the first 150 days of the study, after which the numbers become very small and unstable (Figure 11). The hazard ratio was 0.86. At the 50th percentile level, time to first crisis was 64 days in the L-glutamine vs 44 days in the placebo group, a difference of 20 days.

Figure 11. Time to First SCC – Kaplan Meier Plot - Study 10478



CI = confidence interval, HR = hazard ratio, SCC = sickle cell crisis.
[a] log rank test'

These data were analyzed for statistical significance via Log-Rank test (Table 33).

Table 33. Time to First SCC Log Rank Test - Study 10478

Point Estimate (95% CI) of the Quartiles of the Survival Curve (Days)	Study 10478	
	L-glutamine	Placebo
P-value	0.5861	
75th	169.0 (83.0, --)	220.0 (62.0, --)
50th (Median)	64.0 (15.0, 148.0)	44.0 (6.0, 86.0)
25th	12.0 (1.0, 38.0)	6.0 (2.0, 20.0)

Censoring date is the earlier of the date of taper period start and the study exit date.
CI = confidence interval, SCC = sickle cell crisis.

5.3.5.3 Percentage of Time Hospitalized

The percentage of time hospitalized was analyzed for the ITT population using an ANOVA model with treatment as the main effect (Table 34). The median percentage of time hospitalized was 0% for each group, indicating that at least half of the patients in each group were not hospitalized during the study.

Table 34. Percentage of Time Hospitalized

	Study 10478	
Percentage of Time Hospitalized[a]	**L-glutamine N = 37**	**Placebo N = 33**
P-value	0.8982	
Descriptive statistics		
Mean (SD)	4.3 (8.81)	4.6 (9.89)
Median (min, max)	0 (0, 38)	0 (0, 40)
LS mean (SE)	4.3 (1.534)	4.6 (1.625)
95% CI	1.230, 7.354	1.337, 7.821
LS mean difference (SE)[b]	-0.287 (2.235)	
95% CI	-4.747, 4.173	

CI = confidence interval, LS = least squares, SD = standard deviation.

[a] The percentage of time hospitalized is the cumulative duration of hospitalization divided by the length of time on study times 100.

[b] Difference is L-glutamine minus placebo.

5.4 Discussion and Efficacy Conclusions

The clinical trends observed in the supportive Phase 2 study 10478, including fewer SCCs, hospitalizations, and longer time to first SCC relative to placebo, were promising and supportive of continuing on to a Phase 3 clinical trial. The increased variability in the distribution of the number of SCCs, the relatively small population size of this proof of concept study, and the baseline imbalance in gender and HU use in Study 10478 may have contributed to the lack of statistical significance in the primary efficacy analysis.

Phase 3 Study 09-01was designed based on experience from Study 10478 and additional input from the FDA. In Study 09-01, the analysis of the primary efficacy endpoint using a CMH test with modified ridit demonstrated significantly fewer SCCs overall for the L-glutamine treatment group compared to placebo treatment group over 48 weeks ($p = 0.0052$). The L-glutamine group had 25% fewer events (1 crisis fewer) than the placebo group. The robustness of the results of the primary efficacy analysis was demonstrated via several sensitivity analyses testing the impact of stratification factors, imputation methods, ranking options, and statistical methodology. The results from analyses of multiple other endpoints provide additional, consistent evidence of the benefit of L-glutamine over placebo.

For time to first SCC, the crisis-free survival probability remained greater in the L-glutamine treatment group relative to the placebo group throughout the duration of the study. At the (median) 50^{th} percentile level, time to first crisis was 84 days in the L-glutamine vs 54 days in the placebo group, a difference of 30 days. The hazard ratio for this analysis was 0.69, corresponding to a risk reduction of 31%. Time to second SCC (measured from the beginning of the study) was 212 days in the L-glutamine vs 133 days in the placebo group, a difference of 79 days with a hazard ratio of 0.68. The clinical relevance of the observed difference in frequency of SCC and time to first and second SCC relative to placebo was confirmed by similar differences relative to placebo in number of occurrences of ACS, hospitalizations, time hospitalized, and blood transfusions.

Even a single sickle cell crisis is considered a significant event due to the severity of pain and the impact on quality of life (Hillman et al, 2011; Platt et al, 1991). In our trial, the group of patients treated with L-glutamine experienced 1 fewer crisis, translating to 25% fewer SCCs overall, in a population where 2/3 of patients enrolled were already being treated with hydroxyurea.

In addition, the clinical relevance of the difference in SCCs between groups demonstrated with L-glutamine was supported by additional endpoint analyses of events related to a crisis as it presents clinically. Analyses of the number of hospitalizations for sickle cell pain, days hospitalized, occurrence of ACS, and transfusions all demonstrated that treatment with L-glutamine had a beneficial impact on these disease manifestations in 1 or both studies.

In summary, this clinical development program examined a rare, chronic disease state that is difficult to study due to the severity and nature of the illness and its effect on the disease population (Nottage et al, 2016; Dampier et al, 2013; Crosby et al, 2009; McGrath et al, 2008). The totality of evidence from these studies demonstrated that L-glutamine therapy provided consistent evidence of benefit relative to placebo in the number of SCCs and other events related

to their clinical presentation. These other clinically meaningful events include the number of hospitalizations for sickle cell pain, occurrence of ACS, and time to first and second SCC. The findings for the primary endpoint are robust to alternative imputation and analytical approaches.

6 OVERVIEW OF SAFETY

The L-glutamine clinical development program assessed the safety and tolerability of L-glutamine. Studies 10478 and 09-01 were included in the integrated safety analyses for this submission and are presented as the "Safety Population" in this briefing document. The Safety Population is comprised of 298 patients in Study 10478 and Study 09-01 who received at least 1 dose of study medication.

6.1 Extent of Exposure

6.1.1 Enumeration of Patients

The Safety Population included a total of 298 patients who received at least 1 dose of study medication; 187 patients treated with L-glutamine and 111 patients treated with placebo. Two patients, 1 from each study, were randomized but did not receive study drug and thus are not included in the Safety Population. Overall, 115 patients (61.5%) in the L-glutamine treatment group and 71 patients (64.0%) in the placebo treatment group completed the study. Reasons for discontinuation were similar between treatment groups. The most common reason for discontinuation in both treatment groups was consent withdrawn (26 patients [13.9%] in the L-glutamine treatment group and 14 patients [12.6%] in the placebo treatment group) (Table 35).

Table 35. Summary of Patient Disposition (Safety Population)

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

Studies included: Study 10478 and Study 09-01.
AE = adverse event.
[a] Percent based on total N in group.

6.1.2 Duration of Exposure

An overall summary of exposure data for all patients is presented in Table 36. The mean (standard deviation [SD]) duration of exposure was 268.9 (126.92) days for the L-glutamine treatment group and 283.3 (121.63) days for the placebo treatment group. The majority of patients in both treatment groups received at least 48 weeks of treatment (58.3% in the L-glutamine treatment group and 65.8% in the placebo treatment group). In the L-glutamine and

placebo treatment groups, respectively, 7.0% and 9.0% of patients received ≥ 53 weeks of treatment.

The total exposure to L-glutamine was 137.7 patient-years. A total of 187 patients received L-glutamine for ≥ 1 day, 136 patients received L-glutamine for ≥ 6 months (24 weeks), and 109 patients received L-glutamine for ≥ 1 year (48 weeks).

Table 36. Summary of Drug Exposure (Safety Population)

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

Studies included: Study 10478 and Study 09-01.
SD = standard deviation.
[a] Duration of exposure = last day on study medication – first day on study medication + 1, where last day on study medication includes the taper phase.
[b] Number of patient-years = number of patient-days divided by 365.25.

6.2 Overall Summary of Adverse Events

An overall summary of TEAEs is provided in Table 37. Of note, sickle cell crises were recorded as adverse events and are included in the safety analysis. In both treatment groups, adverse events were consistent with the disease population and duration of the study.

Treatment-emergent adverse events were reported in 96.3% of patients in the L-glutamine treatment group and 97.3% of patients in the placebo treatment group. Treatment-emergent adverse events considered by the investigator to be related to study drug occurred in 18.7% of patients in the L-glutamine treatment group and 13.5% of patients in the placebo treatment group (Table 43). Summaries of deaths, nonfatal serious adverse events (SAEs), discontinuations due to TEAEs, and common TEAEs are presented in the sections below.

Table 37. Overall Summary of AEs (Safety Population)

Parameter	L-glutamine N = 187	Placebo N = 111
Number of events		
Total number of TEAEs[a]	1904	1299
Total number of drug-related TEAEs[b]	77	32
Total number of SAEs	481	411
Total number of drug-related SAEs	5	6
Number of patients with events		
Patients with at least 1, n (%)		
TEAE	180 (96.3)	108 (97.3)
Drug-related TEAE	35 (18.7)	15 (13.5)
SAE	141 (75.4)	89 (80.2)
Drug-related SAE	3 (1.6)	3 (2.7)
Patients who discontinued treatment, n (%)		
Due to TEAE	5 (2.7)	1 (0.9)
Due to drug-related TEAE	3 (1.6)	0 (0.0)
Due to SAE	2 (1.1)	1 (0.9)
Due to drug-related SAE	1 (0.5)	0 (0.0)
Patients who died due to TEAE, n (%)	3 (1.6)	0 (0.0)
Patients who died due to drug-related TEAE, n (%)	0 (0.0)	0 (0.0)

Studies included: Study 10478 and Study 09-01.
AE = adverse event, SAE = serious adverse event, TEAE = treatment-emergent adverse event.
[a] A TEAE is defined as an AE with onset date on or after the first dose of study drug and through 30 days after last dose of study medication.
[b] Drug-related TEAEs are those with relationship to study drug reported as 'possible', 'probable', 'definite', or missing.

6.3 Deaths and Nonfatal Serious Adverse Events

6.3.1 Deaths

There were 3 deaths due to TEAEs in L-glutamine-treated patients (Table 38). None of the deaths were considered by the investigator to be related to study drug. No deaths were reported in the placebo group. One treatment-emergent death occurred in Study 10478 and two occurred in Study 09-01. All patients who died were also receiving HU during the trial. Their ages at the time of death were 37, 46 and 45 respectively. There were no deaths among pediatric patients.

The patient death in Study 10478 was of a 37-year-old woman with SCD. She experienced abdominal pain for which she was advised by the site to seek medical attention which she refused. She was subsequently hospitalized with altered consciousness and hypoglycemia, both deemed unrelated to study drug by the investigator. After being administered 50% dextrose and Narcan (naloxone) and receiving blood and fresh frozen plasma transfusion, she coded 4 times and died due to severe anemia, renal failure, and respiratory failure.

The first patient death in Study 09-01 was of a 46-year-old woman with SCD. She presented at the emergency room with acute SCC. Her sudden death was assessed as severe in intensity and not related to study drug. It was later identified as cardiac arrest, serious, severe, a single episode, and not related to study drug.

The second patient death in Study 09-01 was of a 45-year-old man with SCD. During the study he developed the following 3 SAEs of special interest: acute infarct/transient ischemic attack (resolved with sequelae), acute/chronic renal failure (completely resolved), and cardiac arrest (fatal outcome). All 3 were not considered to be related to study drug. The cardiac arrest resulted in sudden death.

Additional details regarding patient deaths can be found in the narratives provided in Appendix 2.

Table 38. Mortality Events (Safety Population)

Age/Sex/ Treatment	Comorbidities	Event (PT)	Last Dose of Study Medication (Study Day)	Number of Days from Last Dose to Death
37 F L-glutamine	Diabetes Severe anemia Renal failure Respiratory failure	Hypoglycemia Altered State of Consciousness	314	17
46 F L-glutamine	Medical history of jaundice Systolic ejection murmur 2/6	Cardiac Arrest	288	10
45 M L-glutamine	Pulmonary hypertension Renal failure	Cardiac Arrest	318	1

PT = preferred term.

A summary of mortality is presented in Table 39. In this analysis, the crude mortality rate was 1.6% and the exposure-adjusted mortality rate was 2.2 deaths per 100 patient-years. These rates were lower than mortality rates described in published clinical trials that enrolled patients with a history of 2 or more sickle cell crises per year (Steinberg et al, 2003, Ataga et al, 2017), the most appropriate patient population for comparison. In Steinberg et al, the number of deaths per 100 patient-years was 3.1 for patients originally randomized to HU, 3.6 for patients originally randomized to placebo, and 3.3 for the overall population. These rates are all higher than the mortality rate per 100 patient-years seen in our safety population. Ataga et al. was a 1 year study

with a population similar to the L-glutamine safety population in terms of inclusion criteria such as, age, hydroxurea use, and disease severity; this paper analyzed the use of a P-selectin inhibitor (crizanlizumab) to reduce sickle cell crisis. Of the 65 patients in the placebo group of this study, 2 patients died, thus the crude mortality rate was 3.08%. The mortality rate per 100 patient-years can be estimated by assuming that each patient had exposure equal to 1 year (the length of the study), i.e. 65 patient-years of exposure and a mortality rate of 3.08 per 100 patient-years. Note that this is an over-estimate of exposure since 24 patients withdrew early, thus resulting in a conservative estimate of the mortality rate per 100 patient-years. Thus, both the crude mortality rate and the rate per 100 patient-years in the literature for this population are higher than the rates observed in the L-glutamine safety population.

Table 39. Summary of Mortality (Safety Population)

Parameter	L-glutamine (N = 187)
Number of treatment-emergent deaths	3
Crude mortality (%)	1.6
Total exposure in patient-years	137.7
Mortality per 100 patient-years	2.2

Crude mortality = (number of treatment-emergent deaths / number of patients in each group) × 100.
Total exposure in patient-years = summation of all exposure / 365.25, where exposure = last dose date – first dose date + 1.
Mortality per 100 patient-years = number of deaths / total exposure in patient-years × 100.

6.3.2 Nonfatal Serious Adverse Events

A summary of SAEs occurring in ≥ 2% of patients in the L-glutamine treatment group by preferred term (PT) is presented in Table 40. Serious adverse events occurred in 141 patients (75.4%) in the L-glutamine treatment group and 89 patients (80.2%) in the placebo treatment group. The most common SAEs occurring the in the L-glutamine group were sickle cell anaemia with crisis (124 patients [66.3%] in the L-glutamine treatment group and 80 patients [72.1%] in the placebo treatment group), ACS (13 patients [7.0%] in the L-glutamine treatment group and 21 patients [18.9%] in the placebo treatment group), and pneumonia (9 patients [4.8%] in the L-glutamine treatment group and 10 patients [9.0%] in the placebo treatment group).

Table 40. Summary of SAEs Occurring in ≥ 2% of L-glutamine-treated Patients, by PT (Safety Population)

SOC PT	L-glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 SAE	141 (75.4)	89 (80.2)
Sickle cell anemia with crisis	124 (66.3)	80 (72.1)
ACS	13 (7.0)	21 (18.9)
Chest pain	5 (2.7)	2 (1.8)
Pyrexia	5 (2.7)	4 (3.6)
Pneumonia	9 (4.8)	10 (9.0)
Pregnancy	4 (2.1)	3 (2.7)
Asthma	4 (2.1)	3 (2.7)

Studies included: Study 10478 and Study 09-01.
AEs are counted only once per patient within the MedDRA category.
ACS = acute chest syndrome, PT = preferred term, SAE = serious adverse event, SOC = system organ class.

6.4 Discontinuations from the Studies Because of Adverse Events

A summary of the TEAEs that led to withdrawal for patients who discontinued treatment due to a TEAE is presented in Table 37. The proportion of TEAEs leading to withdrawal was slightly higher in the L-glutamine treatment group (2.7%, 5 patients) compared to the placebo treatment group (0.9%, 1 patient). Three patients (1.6%) in the L-glutamine treatment group had at least 1 TEAE leading to discontinuation that was considered by the investigator to be related to study drug; no related events leading to discontinuation were reported in the placebo treatment group. Serious adverse events leading to study drug discontinuation occurred in 2 patients (1.1%) in the L-glutamine treatment group; 1 patient (0.5%) had at least 1 SAE leading to discontinuation that was considered by the investigator to be related to study drug. Serious AEs leading to study drug discontinuation occurred in 1 patient (0.9%) in the placebo treatment group; no SAEs leading to study drug discontinuation were considered by the investigator to be related to study drug.

A summary of TEAEs that led to withdrawal is presented by system organ class (SOC) and PT in Table 41.

Table 41. TEAEs That Led to Withdrawal in 1 or More Patient, by PT (Safety Population)

SOC PT	L-glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 TEAE that led to withdrawal	5 (2.7)	1 (0.9)
Hypersplenism	1 (0.5)	0
Abdominal pain	1 (0.5)	0
Dyspepsia	1 (0.5)	0
Burning sensation	1 (0.5)	0
Pregnancy	1 (0.5)	1 (0.9)
Hot flush	1 (0.5)	0

Studies included: Study 10478 and Study 09-01.
AEs are counted only once per patient within the MedDRA category.
PT = preferred term, SOC = system organ class, TEAE = treatment-emergent adverse event.

6.5 Treatment-Emergent Adverse Events

6.5.1 Common Treatment-Emergent Adverse Events

A summary of TEAEs occurring in ≥ 5% of L-glutamine-treated patients by decreasing frequency of PT is presented in Table 42. Treatment-emergent adverse events of pyrexia (17.1% L-glutamine, 27.9% placebo), ACS (10.2% L-glutamine, 21.6% placebo), and rash (1.6% L-glutamine, 10.8% placebo) occurred at a notably higher incidence in the placebo group than in the L-glutamine group. For all other TEAEs, the proportions of patients reporting events in both treatment groups were similar.

Table 42. Summary of TEAEs, Occurring in ≥ 5% of L-glutamine-treated Patients, by PT (Safety Population)

PT	L-glutamine N = 187 n (%)	Placebo N = 111 n (%)
Patients with at least 1 TEAE	180 (96.3)	108 (97.3)
Sickle cell anaemia with crisis	152 (81.3)	97 (87.4)
Constipation	40 (21.4)	20 (18.0)
Nausea	36 (19.3)	16 (14.4)
Headache	34 (18.2)	17 (15.3)
Pyrexia	32 (17.1)	31 (27.9)
Cough	29 (15.5)	15 (13.5)
Pain in extremity	25 (13.4)	8 (7.2)
Upper respiratory tract infection	25 (13.4)	20 (18.0)
Back pain	23 (12.3)	6 (5.4)
Chest pain	23 (12.3)	9 (8.1)
Vomiting	23 (12.3)	14 (12.6)
Arthralgia	22 (11.8)	15 (13.5)
Abdominal pain	19 (10.2)	10 (9.0)
Abdominal pain upper	19 (10.2)	8 (7.2)
ACS	19 (10.2)	24 (21.6)
Diarrhoea	17 (9.1)	7 (6.3)
Pneumonia	15 (8.0)	14 (12.6)
Ocular icterus	14 (7.5)	10 (9.0)
Nasopharyngitis	13 (7.0)	9 (8.1)
Nasal congestion	12 (6.4)	6 (5.4)
Oropharyngeal pain	12 (6.4)	12 (10.8)
Pruritus	12 (6.4)	9 (8.1)
Urinary tract infection	11 (5.9)	3 (2.7)
Fatigue	10 (5.3)	1 (0.9)

Studies included: Study 10478 and Study 09-01.
AEs are counted only once per patient within the MedDRA category.
PTs are sorted by descending frequency in the L-glutamine group.
ACS = acute chest syndrome, PT = preferred term, TEAE = treatment-emergent adverse event.

Drug-related TEAEs

A summary of the most common drug-related TEAEs, occurring in ≥ 1% of L-glutamine-treated patients, by PT and descending frequency is presented in Table 43. Drug-related TEAEs are those with a relationship to study drug reported as “possible”, “probable”, “definite”, or missing. Drug-related TEAEs were reported by 35 patients (18.7%) in the L-glutamine treatment group and 15 patients (13.5%) in the placebo treatment group. The most commonly reported drug-related TEAE, occurring in > 5% of patients in the L-glutamine group, was constipation (14 patients [7.5%] in the L-glutamine treatment group and 5 patients [4.5%] in the placebo

treatment group). All other drug-related TEAEs in both treatment groups occurred in less than 5% of patients.

Table 43. Summary of Drug-related TEAEs in ≥ 1% of Patients, by PT (Safety Population)

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

Studies included: Study 10478 and Study 09-01.
Drug-related TEAEs are those with a relationship to study drug reported as "possible", "probable", "definite", or missing.
AEs are counted only once per patient within the MedDRA category.
PTs are sorted by descending frequency in the L-glutamine group.
PT = preferred term, TEAE = treatment-emergent adverse event.

Severity of TEAEs

The majority of patients in the L-glutamine or placebo group had TEAEs with a maximum severity of moderate (80 patients [42.8%] and 43 patients [38.7%], respectively) or severe (82 patients [43.9%] and 56 patients [50.5%], respectively), with similar percentages observed between the 2 treatment groups.

The most commonly reported severe TEAE in the L-glutamine group at the PT level was sickle cell anaemia with crisis, with a higher percentage reported in the placebo group compared with the L-glutamine group (47 patients [42.3%] compared with 62 patients [33.2%], respectively).

Of the 35 patients (18.7%) in the L-glutamine group and the 15 patients (13.5%) in the placebo group with drug-related TEAEs (Table 37), a drug-related TEAE with a maximum intensity of severe was reported in 4 patients (2.1%) in the L-glutamine group (events of sickle cell anaemia with crisis, abdominal pain, dyspepsia, and chest pain) and 2 patients (1.8%) in the placebo group (events of abdominal pain, constipation, and back pain).

6.5.2 Adverse Events in Subpopulations

The subgroups of interest for which these safety parameters were evaluated are sex (male, female), age (5 to 12 years, 13 to 18 years, and > 18 years; and ≤ 18 years, > 18 years), race (Black or African American, other), diagnosis (sickle cell anemia, sickle β^0-thalassemia), and HU use at baseline (yes, no).

6.5.2.1 Sex

The most commonly reported TEAE among all patients who received L-glutamine for both male and female patients was sickle cell anaemia with crisis which was reported in a similar percentage of male and female patients in the L-glutamine group and in the placebo group. A higher percentage of female patients in the L-glutamine group reported TEAEs of nausea (28 patients [27.2%]) compared with male patients (8 patients [9.5%]). The percentage of patients reporting all other TEAEs was generally similar between male and female patients for the L-glutamine and placebo groups.

No male patients reported TEAEs that led to withdrawal in the L-glutamine or placebo groups. In female patients, 5 patients (4.9%) in the L-glutamine group and 1 patient (1.7%) in the placebo group reported TEAEs that led to withdrawal.

The percentage of patients who reported SAEs was similar for male and female patients in the L-glutamine and the placebo group. The most commonly reported SAE among all patients who received L-glutamine for both male and female patients was sickle cell anaemia with crisis, which was reported in a similar percentage for female patients across treatment groups (69 patients [67.0%] and 40 patients [69.0%] for L-glutamine and placebo, respectively) and in male patients, was reported in a higher percentage in the placebo group (40 patients [75.5%]) compared with L-glutamine (55 patients [65.5%]).

6.5.2.2 Age

The most commonly reported TEAE among all patients who received L-glutamine in all age groups (age group 1: 5 to 12 years, 13 to 18 years, > 18 years, and age group 2: ≤ 18 years or > 18 years) was sickle cell anemia with crisis, which was reported in a similar percentage of patients in the L-glutamine group and in the placebo group. There were multiple TEAEs where a notable higher percentage of patients reported TEAE among patients ≤ 18 years old compared with patients > 18 years old in the L-glutamine group: ACS (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 patients reporting all other TEAEs was generally similar between the age groups in the L-glutamine group.

Two patients (5.7%) aged 5 to 12 years old, 1 patient (2.2%) aged 13 to 18 years old and 2 patients (1.9%) aged > 18 years old reported TEAEs that led to withdrawal in the L-glutamine group. One patient (1.6%) in the placebo group aged > 18 years old reported a TEAE that led to withdrawal. This translates to 3 patients (3.8%) ≤ 18 years of age and 2 patients (1.9%) > 18 years of age who reported TEAEs that led to withdrawal in the L-glutamine group.

The percentage of patients who reported SAEs was similar for patients who were ≤ 18 years of age and patients who were > 18 years of age in the L-glutamine group and in the placebo group. However, when dividing the younger age group into 2 separate age categories (5 to 12 years and 13 to 18 years) in age group 1, the percentage of patients who reported SAEs in the L-glutamine group was slightly higher in patients who were 13 to 18 years old (39 patients [86.7%]) compared with patients who were 5 to 12 years old (24 patients [68.6%]). The opposite was observed for the placebo group: the percentage of patients who reported SAEs was slightly higher in patients who were 5 to 12 years old (18 patients [94.7%]) compared with patients who were 13 to 18 years old (21 patients [72.4%]).

6.5.2.3 Race

The percentage of patients who reported TEAEs was similar for patients with a race of Black or African American and other in the L-glutamine group and the placebo group. The most commonly reported TEAE and SAE among all patients who received L-glutamine for both patients with a race of Black or African American and other was sickle cell anaemia with crisis, which was reported in a slightly higher percentage of patients with a race of other compared with Black or African American patients in the L-glutamine group. However, it is noted that the small number of patients in the other racial category may have precluded meaningful comparisons between the races.

No patients with a race of other reported TEAEs that led to withdrawal in the L-glutamine or placebo groups. Among Black or African American patients, 5 patients (2.7%) in the L-glutamine group and 1 patient (0.9%) in the placebo group reported TEAEs that led to withdrawal.

6.5.3 Diagnosis

The percentage of patients who reported TEAEs was similar for patients diagnosed with sickle cell anemia and sickle β^0-thalassemia in the L-glutamine group and the placebo group. The most commonly reported TEAE among all patients who received L-glutamine for both diagnosis categories was sickle cell anaemia with crisis, which was reported in a higher percentage of patients with a diagnosis of sickle cell anemia compared with patients with a diagnosis of sickle β^0-thalassemia in the L-glutamine group (140 patients [82.8%] and 11 patients [68.8%], respectively); a similar pattern was seen for the placebo group (87 patients [87.9%] and 10 patients [83.3%], respectively). However, it is noted that the small number of patients who were diagnosed with sickle β^0-thalassemia may have precluded meaningful comparisons between diagnosis categories.

No patients with a diagnosis of sickle β^0-thalassemia reported TEAEs that led to withdrawal in the L-glutamine or placebo groups. In patients with a diagnosis of sickle cell anemia, 5 patients (3.0%) in the L-glutamine group and 1 patient (1.0%) in the placebo group reported TEAEs that led to withdrawal. These events included hypersplenism, abdominal pain, dyspepsia, burning sensation, pregnancy, and hot flush in L-glutamine patients and pregnancy in the placebo patient.

The percentage of patients who reported SAEs was similar for patients with a diagnosis of sickle cell anemia and sickle β^0-thalassemia in the L-glutamine group (128 patients [75.7%] and

13 patients [81.3%], respectively) and was slightly higher in the placebo group (81 patients [81.8%] and 8 patients [66.7%], respectively). The most commonly reported SAE among all patients who received L-glutamine for both diagnosis categories was sickle cell anaemia with crisis which was reported in a similar percentage of patients with a diagnosis of sickle cell anemia and sickle β^0-thalassemia in the L-glutamine group and in the placebo group. As previously noted, the small number of patients with a diagnosis of sickle β^0-thalassemia may have precluded meaningful comparisons between the diagnosis categories.

6.5.4 Hydroxyurea Use at Baseline

The percentage of patients who reported TEAEs was similar for patients with HU use at baseline and patients without HU use at baseline in the L-glutamine group and in the placebo group. The most commonly reported TEAE among all patients who received L-glutamine for patients with or without HU use at baseline was sickle cell anaemia with crisis which was reported in a slightly higher percentage of patients with HU use at baseline compared with no HU use at baseline in the L-glutamine group (107 patients [86.3%] and 45 patients [71.4%], respectively) and in the placebo group (60 patients [92.3%] and 37 patients [80.4%], respectively).

In patients with HU use at baseline, 2 patients (1.6%) in the L-glutamine group and 1 patient (1.5%) in the placebo group reported TEAEs that led to withdrawal. In patients without HU use at baseline, 3 patients (4.8%) in the L-glutamine group reported TEAEs that led to withdrawal. No patients in the placebo group without HU use at baseline reported TEAEs that led to withdrawal.

The percentage of patients who reported SAEs was slightly higher for patients with HU use at baseline compared with patients without HU use at baseline in the L-glutamine group (100 patients [80.6%] and 41 patients [65.1%], respectively) and in the placebo group (57 patients [87.7%] and 32 patients [69.6%], respectively). The most commonly reported SAE among all patients was sickle cell anaemia with crisis, which was reported in a higher percentage of patients with HU use at baseline compared with patients without HU use at baseline in the L-glutamine group (90 patients [72.6%] and 34 patients [54.0%], respectively) and in the placebo group (51 patients [78.5%] and 29 patients [63.0%], respectively).

6.5.5 Safety Data in Patients Exposed to L-glutamine for ≥ 48 Weeks

Of the 298 patients included in the safety populations of both studies, 109 (58.3%) patients received L-glutamine for ≥ 48 weeks. In addition, 13 (7.0%) patients received L-glutamine for ≥ 53 weeks. Although there was no analysis of drug tolerance or withdrawal, there was no suggestion of either of these as potential safety concerns in either study.

6.6 Laboratory Evaluations and Vital Signs

There were no notable differences between treatment groups with regard to clinical laboratory parameters or vital signs. For both the L-glutamine and placebo groups, mean hematology values at end of treatment were generally similar to baseline, with slight changes from baseline observed for most parameters. The baseline values and mean (SD) change from baseline to the end of treatment of BUN and creatinine were also similar between the L-glutamine and placebo

groups. The number of patients with outlying values for ALT, AST, ALT, or AST, and alkaline phosphatase were numerically low and similar between L-glutamine and placebo groups. For the parameter of total bilirubin, the percentage of patients with a total bilirubin value > ULN at any time postbaseline was numerically high (82.0% and 88.4% in the L-glutamine and placebo groups, respectively), but similar between groups.

The mean changes from baseline to end of treatment were generally similar between the L-glutamine and placebo groups. A slightly greater mean increase was observed in the L-glutamine group compared with placebo for systolic blood pressure (1.84 mmHg vs -0.14 mmHg) and pulse rate (2.90 beats per minute [bpm] vs 1.11 bpm).

6.7 Safety in Special Populations

6.7.1 Sex, Age, Race, Diagnosis, and Hydroxyurea Use

As described in Section 6.5.2, there were no notable differences observed by sex, age, race, diagnosis, and HU use in the percentage of patients who reported TEAEs, SAEs, or TEAEs that led to withdrawal in the L-glutamine or placebo group. Both Study 10478 and Study 09-01 allowed for inclusion of patients who were at least 5 years of age. Across both studies, a total of 54 patients (18.1%) were 5 to 12 years old and 74 patients (24.8%) were 13 to 18 years old. Overall, there were no notable differences in the percentage of TEAEs in pediatric patients as compared with adult patients in Study 10478 and Study 09-01.

6.7.2 Use in Pregnancy and Lactation

Animal reproduction studies have not been conducted with L-glutamine. It is also not known whether L-glutamine can cause fetal harm when administered to a pregnant woman or whether L-glutamine can affect reproductive capacity. It is not known whether L-glutamine is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when L-glutamine is administered to a nursing woman.

6.8 Drug Interactions

No PK studies that examined the interaction between L-glutamine and drugs commonly used by patients with SCD were reported in the literature. Patients with SCD are often treated with HU to promote growth of fetal hemoglobin, receive analgesics and opioids for pain control, receive blood transfusions for anemia, and treated with antibiotics for infections and iron chelation therapy for iron overload (Yawn et al, 2014).

Several published nonclinical studies have examined the effects of combining L-glutamine with other agents such as: indomethacin, (Arndt et al, 1999) cyclosporine, (Zhang et al, 1995) methotrexate, (Fox et al, 1988) 5-fluorouracil, (Jacobs et al, 1987) and dexamethasone (Boza et al, 2001). In these studies, L-glutamine treatment did not cause any detrimental effects and generally alleviated deleterious side effects normally caused by these agents. Because metabolism of glutamine is mediated via non-cytochrome P450 (CYP) enzymes, glutamine pharmacokinetics are unlikely to be affected by other agents through CYP enzyme inhibition or induction.

6.9 Postmarketing Data

From 01 Aug 2008 to 10 Jun 2016, a total of 190,854 packets of NutreStore® were distributed. The NutreStore® dosage is 30 gram/day for 16 weeks; therefore, this equates to 284 courses of treatment with NutreStore®. No AEs for patients taking NutreStore® were reported during the entire postmarketing reporting period. Literature reports published during the postmarketing period did not include any reports of individuals who experienced AEs related to L-glutamine administration during the studies.

6.10 Safety Conclusion

The clinical development program established the safety of L-glutamine 0.3/kg bid for up to 48 weeks in adult and pediatric patients. Overall, L-glutamine was well tolerated. The integrated safety data demonstrated that oral L-glutamine at 0.3 gram/kg bid had a safety profile similar to placebo. Serious events were common in both treatment groups and consistent with the underlying disease. The most common TEAEs occurring in ≥ 10% of patients in the L-glutamine group and with greater frequency than placebo included constipation, nausea, headache, cough, pain in extremity, back pain, chest pain, abdominal pain, and abdominal pain upper.

7 BENEFIT/RISK ASSESSMENT AND CONCLUSIONS

7.1 Summary of Benefit

- Sickle cell disease is a devastating, rare, hereditary disease associated with profound clinical manifestations, and shortened lifespan.
- In Phase 3 Study 09-01, relative to placebo, the L-glutamine therapy group had:
  - Lower frequency of SCC by 25% (p=0.0052)
  - Decrease in ACS by 67% (p =0.0028)
  - Decreased frequency for hospitalization by 33% (p =0.0045) and overall shorter stay by 41% (p =0.022)
  - Longer median time to first crisis by 30 days with a hazard ratio of 0.69 (p=0.0152) and second crisis by 79 days with a hazard ratio of 0.68 (p =0.026)
  - Fewer blood transfusion events per patient by 39%
- Effect modifier analysis demonstrates that L-glutamine is effective:
  - In those patients already treated with HU
  - In adults and pediatric patients

7.2 Summary of Risks

Overall, L-glutamine was well tolerated. The integrated safety data demonstrated that oral L-glutamine at 0.3 g/kg bid had a safety profile similar to placebo. Specifically, the clinical development program established the safety of L-glutamine 0.3 g/kg bid for up to 48 weeks for the treatment of SCD in adult and pediatric patients.

7.3 Conclusion

The efficacy findings from 1 pivotal Phase 3 study and 1 supportive adequate and well-controlled Phase 2 study demonstrate that L-glutamine therapy resulted in a significant statistical and clinical difference in the number of SCCs and other events related to the clinical presentation of SCD. Patients receiving L-glutamine therapy experienced 1 fewer SCCs over 48 weeks compared to placebo, which translated into at least 25% fewer SCCs compared to placebo. This treatment is likely to make a meaningful difference in the clinical status, and quality of life of SCD patients affected by severe pain events. According to epidemiological data, this may also lead to longer survival. (Platt et al, 1991; Hillman et al, 2011). The clinical relevance of this difference in SCCs relative to placebo was reinforced by supporting analyses showing a beneficial impact of L-glutamine treatment on other aspects of disease manifestation, such as hospitalizations for sickle cell pain, occurrence of ACS, and time to first SCC.

The integrated safety data demonstrated that oral L-glutamine at 0.3 g/kg bid had a safety profile similar to placebo. Specifically, the clinical development program established the safety of L-glutamine 0.3 g/kg bid for up to 48 weeks for the treatment of SCD in adult and pediatric patients. There is no known safety risk associated with this drug. This is further evidenced by the postmarketing use of L-glutamine for another indication.

The difference in the number of SCCs relative to placebo with a safe and easy to administer oral drug gives L-glutamine a positive benefit/risk ratio. L-glutamine may fill the need for a new standard of care treatment for pediatric patients and increase compliance in adults who have struggled with serious toxicities associated with HU use. Overall, L-glutamine as a treatment for SCD patients is safe and effective. The benefits of the L-glutamine treatment substantially outweigh the risks. These results accentuate the increase in quality of life and decrease in human clinical and health care system utilization for patients with SCD when treated with L-glutamine.

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APPENDIX 1. DETAILS OF STATISTICAL ANALYSES IN STUDY 09-01

LIST OF ABBREVIATIONS

ACS	Acute chest syndrome
ANOVA	Analysis of variance
CI	Confidence intervals
CMH	Cochran-Mantel-Haenszel
CSR	Clinical study report
ER	Emergency room
FAS	Full analysis set
HU	Hydroxyurea
ITT	Intent-to-treat
LOCF	Last observation carried forward
SCC	Sickle cell crisis

1 PRIMARY STATISTICAL ANALYSES IN STUDY 09-01

Table 44 summarizes the analyses of the primary efficacy endpoint. The primary analysis utilized a Cochran-Mantel-Haenszel (CMH) test to evaluate the difference between the L-glutamine and placebo groups. The pre-defined definitive analysis utilized the CMH test with the modified ridits scoring option to adjust for stratum sample size (SCORES=MODRIDIT). Other analyses of the primary endpoint were also run as sensitivity or additional analyses on the intent-to-treat (ITT) population.

Table 44. Analyses of the Primary Efficacy Endpoint

Analysis	Purpose	Analysis
NDA	To use the same inference procedure for estimating the study sample size and for the final analysis	CMH test using modified ridits
Sensitivity	To test effect of stratification factors	CMH test using modified ridits controlling only for region or HU use or omitting both randomization stratification factors
Sensitivity	To test effect of imputation methods	CMH test using modified ridits with missing data imputed by LOCF[a] and time-adjusted LOCF[b]
Sensitivity	To test the ranking options and determine whether results differed based on the ranking method	CMH test run with ranks determined before analysis
Sensitivity	To test statistical methodology and determine whether results differed when using a method specifically intended for count data that did not require imputation	NBR

CMH = Cochran-Mantel-Haenszel, HU = hydroxyurea, LOCF = last observation carried forward, NBR = negative binomial regression.

[a] The number of crises at the time of early discontinuation was used in the Week 48 analysis.

[b] The number of crises at the time of early discontinuation divided by the number of days on study medication and multiplied by 336 (48 × 7 = 336) was used to extrapolate the number of crises per 48 weeks.

1.1 CMH Analysis of Primary Efficacy Endpoint Using Modified Ridits

A CMH test using modified ridits (option SCORES=MODRIDIT) including the randomization stratification factors in the model (HU use and region) was used to analyze the primary endpoint in the new drug application (NDA). This approach is consistent with the method used to estimate the study sample size. The p-value and descriptive statistics were reported.

The following were run as sensitivity analyses for the primary endpoint:

- CMH test using modified ridits omitting the stratification factors (region, HU use, or both), and
- CMH test using modified ridits with missing data imputed by:

- last observation carried forward (LOCF)
- time-adjusted LOCF

1.2 Negative Binomial Regression Analysis of the Primary Efficacy Endpoint

While the number of SCCs may be considered ordinal categorical data suitable for the CMH test, they are more accurately described as count data. Since the negative binomial regression (NBR) analysis is specifically intended for count data this analysis was also performed as a sensitivity analysis of the statistical methodology (Hilbe, 2011).

In analyzing the data using NBR, the number of events over time on study was modeled as a linear function of the predictor variables (the 2 treatments: L-glutamine and placebo), the randomization stratification factors as main effects, and the log of the duration on study as offset. The NBR model was selected because of the following desirable properties for event data:

- Accommodates skewed data: The negative binomial error term of an NBR model allows the variability of the fitted values to increase as the fitted values increase. Because count data is usually skewed (many smaller values and few large values), the model required the flexibility to capture this variability.
- Imputation is not needed: An NBR model with the log of the time on study as an offset term allows the data from patients who withdrew before Week 48 to be used without data imputation because it takes duration of time on study into account thereby transforming the number of events into rates. This also allowed the assessment of whether the primary analysis results would be consistent in a model that does not require imputation.
- An estimate of treatment effect is provided: In addition to providing a p-value for the test of H_0: no difference between L-glutamine and placebo, an NBR model provides an estimate of the treatment effect (rate ratios with confidence intervals [CIs]) converted into an event rate per 48 weeks, which allow an assessment of the clinical significance of the results as opposed to statistical test significance alone.

1.3 Additional Efficacy Endpoints

1.3.1 Time to First SCC

The time to the first SCC (occurring before the start of the tapering period) was calculated in days (date of first SCC - Day 1 date + 1). Patients with no SCCs were censored, with their censoring date set to their taper period start or last date on study medication (whichever was earlier). The estimated survival curve for the time to the first SCC was plotted by treatment and study using the Kaplan-Meier method. The quartiles of this survival curve, point estimates and 95% CIs were reported by treatment and study. A Log-Rank test for the null hypothesis (H_0 = no difference in the survival curves between L-glutamine and placebo treatment groups) was performed for each study.

1.3.2 Occurrences of Acute Chest Syndrome

The number of occurrences of acute chest syndrome (ACS) prior to taper was analyzed using CMH test with modified ridits, including the study-specific randomization stratification factors. The p-value for null hypothesis (H_0 = no difference between treatments) and descriptive statistics were reported.

1.3.3 Numbers of Hospitalizations and Emergency Room Visits

The numbers of hospitalizations and emergency room visits for sickle cell pain through Week 48 were analyzed using the CMH test using modified ridits (option SCORES=MODRIDIT) including the randomization stratification factors in the model (HU use and region). Hospitalizations and ER visits for sickle cell pain must have occurred prior to taper but were not required to be associated with an official SCC to be included. The p-value for null hypotheses (H_0 = no difference between L-glutamine and placebo treatment groups) and descriptive statistics were reported by study.

1.3.4 Cumulative Days in Hospital

Time (cumulative days) in the hospital was analyzed in the CSR for difference between the treatment groups. Time was calculated within patient as the summation of all hospital stays (departure date – registration date +1). If a hospitalization started before tapering but extended past this point all days were included; hospitalizations that began after tapering were excluded.

Summary statistics n, mean, standard deviation, median, minimum and maximum were calculated. The data were analyzed using the non-parametric Wilcoxon rank-sum test.

1.3.5 Hematologic Parameters

Hemoglobin, hematocrit, and reticulocyte counts were measured in both studies at Weeks 0 (baseline), 4, 8, 12, 16, 20, 24, 32, 40, and 48. The change from baseline was analyzed with a repeated measures analysis of variance (ANOVA) model consisting of treatment, patient within treatment, and week main effects; a treatment by week interaction term; and baseline as a covariate. Observed cases were analyzed with no imputation for missing data.

The least squares (LS) mean and 95% CI for each treatment at each week and for the difference between treatments at each week were reported by study. The corresponding p-values for the difference between treatment groups and the difference between treatment groups by week were presented by study.

1.3.6 Blood Transfusions

At the request of the FDA, summaries of packed red blood cell (PRBC) transfusions, simple and exchange, were provided. The verbatim terms from the Blood Products case report form were classified as either exchange or PRBC based on clinical review. For each type of transfusion, the total number of transfusions, number of patients with one or more transfusion, and the number of transfusions per patient were summarized descriptively by treatment group.

APPENDIX 2. NARRATIVES OF DEATHS

Study: 10478

A 37-year-old woman with sickle cell anemia had a history of avascular necrosis of both hips, acute renal failure, aplastic crisis, hemochromatosis, and ongoing liver insufficiency (controlled), intermittent seizures (controlled), pulmonary hypertension (controlled), ankle edema, mild icteric sclerae, right toe numbness, systolic murmur, and bilateral edema of the lower extremities. She was hospitalized on Study Day 332 after 2 days of abdominal pain. The site had been in contact with the patient via telephone, and had encouraged her to seek medical attention 2 days prior to her admission. The patient stated that she did not want to seek medical attention because a hospitalization would conflict with upcoming plans. She was hospitalized with altered consciousness and hypoglycemia. She was given 50% dextrose and Narcan (naloxone) and was transfused with blood and fresh frozen plasma. She was coded 4 times and died due to severe anemia, renal failure, and respiratory failure Study Day 332. The investigator considered the altered consciousness and hypoglycemia to be severe and unrelated to the study drug.

The collected baseline measurement levels: creatinine = 0.7 and albumin = 2.9. The normal ranges for these measurements: creatinine = 0.4 - 1.3 and albumin = 3.5 - 5.8.

Study: 09-01

A 46- year-old black woman with sickle-cell anemia, height of 166 cm and weight of 75.2 kg at screening, had a medical history of jaundice and systolic ejection murmur 2/6. The patient was previously treated with Hydroxyurea 1500 mg PO QD for 18 years. She developed 2 sickle-cell crises within the last year. On Study Day 297, the patient presented to the emergency room due to acute sickle cell crisis, where she suddenly died. Cardiopulmonary resuscitation (CPR) was unsuccessful.

Initially, the cause of death was not identified (stated as "unknown"). This unspecified SAE was reported as: sudden death, severe in intensity, not related to study drug, treated with CPR, and resulted in a fatal outcome. The investigator commented: "Patient arrived at ER (emergency room). CPR in progress. Initial assessment: patient was not verbal and cool to touch." This SAE was later identified as: Cardiac arrest, serious, severe, a single episode, not related to study drug, treated with concomitant medication(s) and procedure(s), and resulted in a fatal outcome.

This patient also developed the following SAE while in the study: Vaso-occlusive crisis from Study Day 237 to Study Day 240, severe in intensity, a single episode, not related to study drug, treated with concomitant medications (Dilaudid IV, Lovenox IV, Oxygen, Potassium Chloride PO, Magnesium PO, and Levaquin IV) and procedure(s), and resolved completely. During the course of the study, the patient also experienced one non-serious AE: Lightheadedness from Study Day 5 to Study Day 28, mild in severity, intermittent, not related to study drug, not treated, and resolved completely.

The collected baseline measurement levels: creatinine = 0.53, INR = 1.2 and albumin = 4.8. The normal ranges for these measurements: creatinine = 0.51 – 0.95, INR = 0.8 – 1.2 and albumin = 3.2 – 5.5.

Study: 09-01

This 45-year-old black man with sickle-cell anemia and a complicated medical history experienced a sudden death on Study Day 318. The last documented day when the patient was still taking study drug in accordance with the protocol requirements was Study Day 303.

During the study, this patient developed the following SAEs of special interest:

1. Acute infarct/ TIA (transient ischemic attack) from Study Day 70 to Study Day 79 (with the same hospitalization dates), moderate in severity, not likely related to study drug, treated with concomitant medications, and resolved with sequelae;
2. Acute/chronic renal failure from Study Day 109 to Study Day 115 (with the same hospitalization dates), severe in intensity, not related to study drug, treated with concomitant medications, and resolved completely;
3. Cardiac arrest on Study Day 318, severe in intensity, a single episode, not related to study drug, treated with concomitant medication(s), and resulted in a fatal outcome.

Besides these 3 SAEs of special interest, the patient also developed another 3 SAEs while in the study: 1) vaso-occlusive crisis, 2) vaso-occlusive crisis, and 3) slurred speech. During the course of the study, the patient experienced the following non-serious AEs: worsening of anemia, dehydration, coagulopathy, hypotension, severe anemia, MRSA (Methicillin-Resistant Staphylococcus Aureus), and left knee swelling.

The collected baseline measurement levels: creatinine = 2.81, INR = 1.2 and albumin = 3.8. The normal ranges for these measurements: creatinine = 0.67 – 1.17, INR = 0.8 – 1.2 and albumin = 3.2 – 5.5.