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A Phase 1/2 Trial of HQK-1001, an Oral Fetal Globin Inducer, in Sickle Cell Disease

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Abstract

Therapeutics which reduce the pathology in sickle cell syndromes are needed, particularly non-cytotoxic therapeutics. Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) is established as a major regulator of disease severity; increased HbF levels correlate with milder clinical courses and improved survival. Accordingly, sodium dimethylbutyrate (HQK-1001), an orally-bioavailable, promoter-targeted fetal globin gene-inducing agent, was evaluated in a randomized, blinded, dose-ranging Phase I/II trial in 24 adult patients with HbSS or S/β thalassemia, to determine safety and tolerability of three escalating dose levels. The study therapeutic was administered once daily for two 6-week cycles, with a 2-week interim dose holiday. Twenty-one patients completed the study. Five patients received study drug at 10 or 20 mg/kg doses, seven patients received study drug at 30 mg/kg/dose, and 4 patients received placebo. HQK-1001 was well-tolerated with no unexpected drug-related adverse events; a dose-limiting toxicity was not identified. Plasma drug levels were sustained above targeted levels for 24 hours. Increases in HbF above baseline were observed particularly with 30 mg/kg/day doses; in five of seven treated patients, a mean absolute increase in HbF of 0.2 g/dl and a mean increase in total hemoglobin (Hgb) of 0.83 g/dl above baseline were observed, whereas no increases occurred in placebo-treated controls. These findings of favorable PK profiles, tolerability, early rises in HbF and total Hgb indicate that trials of longer duration appear warranted to more definitively evaluate the therapeutic potential of HQK-1001 in sickle cell disease.

INTRODUCTION

Sickle cell disease, characterized by HbS ($\alpha_2\beta^S_2$), is a genetic blood disorder which is recognized by WHO as a global health burden.[1] Sickle cell disease causes hemolytic anemia, vaso-occlusion, and cell adhesion, producing widespread organ damage and early mortality.[2-3] Fetal globin, or HbF ($\alpha_2\gamma_2$), has been established as the major modulator of disease severity in biochemical, epidemiologic, and clinical trials.[4-22] While HbF levels >8.6% correlate with improved survival, levels >20% are associated with amelioration of most complications.[8-14,17-18,21-23] One therapeutic, hydroxyurea, is approved for treatment of sickle cell disease and confers considerable benefit in some patients; reduced frequency

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of clinical events and improved survival in adult patients are associated with rises in absolute HbF levels > 0.5 g/dl.[17-18,20] Additional therapeutics that induce HbF expression by different mechanisms are needed for many patients to achieve levels of HbF which are disease-modifying.[4,23]

Previously, two other classes of therapeutics have produced high HbF levels in adult sickle cell patients: demethylating agents (5-azacytidine and decitabine) and short chain fatty acids, of which some are histone deacetylase inhibitors, such as arginine butyrate. [24-26] Clinical trials of these agents resulted in a mean 3-fold increase in HbF, to levels of 18-30%, in adult patients with an associated rise in total hemoglobin of 1 gm/dl above baseline. [24-28] However, these agents require parenteral administration, while oral therapeutics are more feasible and preferable for long-term use. Sodium 2,2 dimethylbutyrate (SDMB, HQK-1001) is a short-chain fatty acid derivative which induces expression of the γ–globin gene promoter in reporter gene assays, γ–globin mRNA and HbF in cultured erythroid progenitors, and of HbF in anemic baboons.[29-32] SDMB is orally bio-available with a half-life of several hours in non-human primates and in normal human volunteers.[30,33] A dose-ranging trial to evaluate safety, tolerability, and pharmacokinetics in normal human volunteers demonstrated no significant drug-related adverse events, and produced pharmacokinetic profiles which are favorable for once-daily dosing, with a $t_{1/2}$ of 9-11 hours at doses projected to be required for therapeutic induction of fetal globin.[33] Accordingly, a blinded, sequential dose-escalating safety and tolerability study of sodium 2,2 dimethylbutyrate (HQK-1001) was performed in patients with sickle cell disease. The drug was well-tolerated, and early signals of induced fetal globin expression were observed in some subjects during this short-term drug administration.

MATERIALS AND METHODS

The primary objective of this trial (NCT00842088) was to determine the safety and tolerability of HQK-1001 administered for 12 weeks at three dose levels in subjects with sickle cell disease. Secondary objectives were to evaluate the pharmacokinetic (PK) profiles of the drug with repeated doses, to assess potential pharmacodynamic effects on fetal globin expression within the relatively brief dosing period, and to identify dose regimens and schedules suitable for further evaluation in follow-on trials. The design was a randomized, blinded, placebo-controlled, sequential dose-escalating trial and was conducted in seven clinical sites, six in the United States and one in Jamaica, with approval of their Investigational Review Boards. Eligibility criteria required that subjects have an established diagnosis of Hb SS or sickle/β-thalassemia, were ages 12-60 years inclusive, and had a history of an average of one significant sickle cell-related medical event requiring medical attention per year for in the preceding three years, or at least one hospitalization for acute chest syndrome in the previous five years. Concomitant hydroxyurea therapy was allowed, if a stable dose was established in the subject for at least six months. A baseline screening HbF level >2% was required, as this threshold was necessary for responses to arginine butyrate in previous trials.[26] Exclusion criteria included a red blood cell transfusion within 3 months before beginning drug or participation in a regular transfusion program, acute vaso-occlusive events within 3 weeks, a history of >4 hospitalizations for sickle cell related events in the preceding 12 months, renal or hepatic compromise, or pulmonary hypertension requiring oxygen therapy. Randomization to active treatment or placebo was assigned according to a table generated by random numbers prepared by biostatisticians in an independent contract research organization, Quintiles, Inc., which monitored the study.

The study drug was administered once-daily for 2 cycles of 6 weeks each. Because arginine butyrate was more effective when administered in an intermittent or pulsed fashion, a 2-week dose holiday was provided between the two treatment cycles.[26] Initial doses were

administered with observation in a clinical research unit. Cohorts of 10, 20, and 30 mg/kg/ doses included at least one placebo subject and six or seven active treatment subjects, and dose escalation between cohorts was allowed sequentially after review of the first four subjects' courses by an independent Safety Monitoring Committee. Patients received supplements with folic acid, 1 mg/day orally, and ferrous gluconate if their ferritin was <1000ng/ml, to support erythropoiesis, as previously shown to be necessary for effective HbF induction.[34] Safety monitoring by blinded investigators included physical examinations, laboratory studies of hematologic, renal, and hepatic function every two weeks, periodic coagulation analyses, electrocardiograms, monthly pregnancy tests, and adverse event monitoring every two weeks during the dosing period and for four weeks after dosing was completed.

HbF was assayed by HPLC at a central reference laboratory by blinded personnel, and F-cells, proportions of red blood cells containing HbF, was assessed by flow cytometry also by blinded personnel. Samples were collected for PK profiles on day 6; drug levels were assayed by previously established methods.[33] Pharmacokinetic parameters which were analyzed included area under the curve from time 0 to last quantifiable concentration ($AUC_{(0\text{-}lqc)}$), AUC from time 0 to infinity ($AUC_{(0\text{-}\infty)}$), maximum concentration of drug (C_{max}), minimum concentration of drug (C_{min}), average concentration of drug (C_{avg}), time of maximum concentration (T_{max}), elimination half-life ($T_{1/2}$), total body clearance (CL/F), and elimination rate constant (λ_z). Pharmacokinetic analyses were performed using WinNonLin^R Professional version 5.2 (Parsight Corp., Mountain View, CA.), as previously described.[33] Descriptive statistics were prepared and statistical analyses were performed **by J.M. White Inc.,** using Statistical Analysis Software (SAS) version 9 or higher. Adverse events were characterized by dose level or placebo using MedDRA version 9 preferred term coding, and laboratory abnormality severity was graded using NCI CTC version 3. All 24 subjects who enrolled received at least one dose of the study drug, and all were included in the safety analysis. The blinded principle investigator (PI) at each site determined **adverse event** (AE) relationship to study drug.

RESULTS

Thirty patients were screened for eligibility criteria; twenty-four subjects met the required criteria, signed informed consent, and were enrolled. Demographic characteristics and baseline hematologic features of the study participants are shown in Table I. Patients ranged in age from 14 to 57 years; the mean ratio of males to females was 0.6 in the four cohorts. The subjects' genotypes were homozygous Hb SS except for **one patient who had HbS-β^+ thalassemia**. No apparent differences in demographic characteristics of age, ethnicity, or baseline height and weight were observed across treatment groups. The patients were of Jamaican or African American ethnicity. All subjects had significant past medical histories characteristic of sickle cell disease, including vaso-occlusive crises, osteonecrosis, pulmonary hypertension, cholelithiasis, priapism, proliferative retinopathy, and joint replacement for avascular necrosis. Twelve subjects had concomitant hydroxyurea (HU) use, including 75% of placebo subjects, and in 57, 50, and 29% of the 10, 20, and 30 mg/kg dose cohorts, respectively. Baseline median HbF levels were 14.5, 7.7, 11.2, and 10.8% in the placebo-treated, 10, 20, and 30 mg/kg dose cohorts, respectively. Mean baseline HbF was in those subjects receiving treatment with HU was 14.4% and in subjects not receiving HU was 7.6%. Baseline total hemoglobin (Hgb) levels ranged from 5.9 to 11.8 g/dl; the means at baseline were 8.8, 8.4, and 9.7 g/dl in the 10, 20, and 30 mg/kg dose 1001-treated cohorts respectively and 9.3 g/dl in the placebo treated group. Baseline total Hb and % HbF in individual subjects by assigned treatment or placebo group and HU use are shown in Table Ib.

Twenty-one patients completed the study and are included in the pharmacodynamic data analysis, all patients were included in the safety analysis, and the drug was well-tolerated. Reasons for early termination included development of more severe anemia (in association with a port infection) which required transfusion within one week after starting the study, and two study discontinuations unrelated to the study drug (inability to continue clinic visits due to work obligations in one subject and hypersensitivity to a concomitant medication in another). There were no unexpected, drug-related serious adverse events (SAEs). However, nine subjects experienced 19 SAEs, summarized in Table 2A. These included 6 subjects receiving active treatment who had 13 SAEs, and 3 placebo-treated subjects who had 6 SAEs. All the SAEs were considered to be expected sickle-related events, including vaso-occlusive crises, an indwelling port infection, worsening anemia (associated with the port infection), arthralgia, and extremity pain. One vaso-occlusive crisis in a placebo-treated subject was classified as possibly drug-related by the blinded investigators. No differences were detected in rates or severity of adverse events between patients who were or were not receiving concomitant HU. Adverse events which were graded as mild to moderate and reported in at least 10% of the subjects are shown in Table 2B; the most common events included vomiting, abdominal pain, arthralgia, bone pain. Other adverse events considered to be treatment-related included nausea and rash in two subjects. Minor transient laboratory abnormalities in liver function tests occurred in four subjects; increases in transaminases were graded as mild (Grade 1) or moderate (grade 2), and all returned to normal before the end of the dosing period. The highest elevation in ALT was 256 (range 68-256) and in AST was 169 U/L (range 73-169). Mild leukopenia occurred in one patient, in whom a decline in WBC from 4.4 to 3.1 × 10^9/L occurred transiently, with subsequent increase to 5.6 × 10^9/L without any change in study drug. There were no episodes of neutropenia or thrombocytopenia. No dose-dependent pattern was observed for incidence and severity of AEs, and there was no clear difference in the incidence and severity of AEs between the HQK-1001 groups compared to placebo treated subjects, **or to HU use,** in this small, dose-ranging study. Arthralgia was reported more frequently in the 30 mg/kg group, while bone pain, cough, and headache were more often reported in the 20 mg/kg group, and pain in an extremity was seen more frequently in the 10 mg/kg group.

Pharmacokinetic studies demonstrated mean PK profiles with dose-dependent increases in AUC and C_{max} of HQK-1001 (Figure 1), with plasma concentrations sustained for >10 hours above concentrations (12 to 71 μg/mL) which induce HbF in erythroid progenitors *in vitro*.[29] Mean maximum concentration increased as dose increased, ranging from 54.9 to 114.0 μg/mL over the 10 to 30 mg/kg dose range. The mean T_{max} ranged from 2.0 to 2.4 hours. Mean minimum concentration at 24 hours was 15.5 μg/mL at 10 mg/kg, 17.8 μg/mL at 20 mg/kg, and 17.6 μg/mL at 30 mg/kg, which are HbF-inducing “therapeutic” levels in erythroid progenitors *in vitro*. The mean AUC_{inf} ranged from 722 at 10 mg/kg to 1302 h*μg/mL at 30 mg/kg. Mean CL/F values increased from 0.015 at 10 mg/kg to 0.026 L/h/kg at 30 mg/kg. However, the mean terminal phase half-life for HQK-1001 decreased from 13.8 at 10 mg/kg to 8.4 hours at 30 mg/kg. The decrease in exposure with increase in dose could possibly be due to an increase in the intrinsic clearance. These studies confirm that this agent has more favorable PK profiles compared to prior-generation butyrates, which are rapidly metabolized and required significantly higher doses administered by infusions or multiple doses, three times/day, such as arginine butyrate and phenylbutyrate.[26,35]

Increases in median percent HbF, absolute HbF, and total hemoglobin on the last observation at Day 97 were observed in at least half of the subjects in the 20 and 30 mg/kg dose cohorts, while no increases were detected in the placebo group. Increases in % HbF of > 1.1% above subjects’ baseline (which are reliably detected in the testing laboratory) were observed at the end of the treatment period in 2/5 subjects treated with 10 mg/kg/dose (by 2.6 and 3.1% above baseline), in 3/5 patients treated at 20 mg/kg doses (by 1.6 to 3% above

baseline), and in 5/7 patients treated with 30 mg/kg doses, (by 1.1 to 3.7% above baseline). Individual responses are shown in Figure 2A. Absolute HbF also increased in the 30 mg/kg dose cohort in five of seven treated subjects; the median increase was 1.82 g/dl, compared to no increase in the 4 placebo-treated subjects. The per cent of F-cells increased from baseline to day 97 by a median of 4.7 %, 0.45%, and 6.1% in the 10,20,and 30 mg/kg 1001 dose cohorts respectively; in the placebo group, F-cells declined by −0.6%. A mean increase in total Hgb of 0.83 g/dl above averaged baseline levels was observed in the 30 mg/kg dose cohort (Figure 2B). Increases in HbF occurred in subjects both with and without concomitant hydroxyurea therapy.

DISCUSSION

Sickle cell disease, a WHO-designated global health burden, still causes significant pain and disability in many patients, despite the beneficial effects of hydroxyurea in approximately half of treated adult patients, and higher responses in most children.[15-21] Elevation of fetal hemoglobin in sickle cell disease is well-established as a modulator of disease severity in sickle cell disease, and agents which can be used without added toxicities with hydroxyurea are needed for many patients.[4,12,23] Sodium dimethylbutyrate, an orally bioavailable, short chain fatty acid derivative fetal globin gene inducer was well-tolerated in this Phase I/II trial. Pharmacokinetic studies demonstrated profiles suitable for once daily dosing, which is preferable over multiple daily dosing in a treatment requiring long-term use.

Dose-limiting toxicity was not identified at the dose levels studied here. HU trials typically demonstrate increases in HbF after 6 months of treatment, and HbF responses in adult patients in the MSH trial averaged 3.5% above baseline.[19] Achieving HbF levels > 0.5 g/dl is associated with increased survival in long (9 and 17 year) follow-up studies of HU in adult sickle cell patients.[17-18]

This dose-ranging safety trial was not designed, dosed for an adequate duration of time, or powered to detect changes in HbF. However, some increases in HbF were detected in subjects treated with the study drug and the findings suggest a trend of increasing response rates and a higher magnitude of effect on HbF and total Hb as the dose was increased. The early signals in HbF observed in this safety study, particularly at the 30 mg/kg/dose level, are therefore encouraging, and also suggest that higher HbF levels may be inducible with longer duration of administration.

Sickle cell disease is characterized by anemia related to short red cell lifespan, intravascular hemolysis, and inappropriately low levels of erythropoietin for the degree of anemia.[2-3,11,35] The anemia typically becomes more severe with increasing age, perhaps related to renal compromise and reduced erythropoietin levels. This HbF-inducing therapeutic candidate, HQK-1001, was selected for clinical development for its improved PK profile in non-human primates compared to prior generation butyrates, and a second activity of increasing erythroid cell proliferation through STAT-5 signaling and enhanced Bcl-xL expression, *in vitro* and *in vivo*, in anemic baboons.[29-31] Demonstration of any erythropoietic effect was not expected over the brief time-frame of this study in sickle cell disease, in the presence of markedly reduced red blood cell lifespan of 16 days, so it is encouraging that increases in total hemoglobin (mean rise of 0.8 g/dl) occurred in the 30 mg/kg dose cohort. It is also interesting that changes in total Hgb were observed to a lesser extent in subjects receiving concomitant hydroxyurea treatment, as a proliferative effect would be expected to be inhibited by the cytostatic effects of hydroxyurea.

Steinberg and others have strongly recommended that a combination therapeutic approach is needed to modify clinical disease in many patients with sickle cell disease, as multiple

agents acting by different mechanisms are usually required for effective control of most systemic medical conditions, and differences in drug metabolism often render response rates to any drug 25-40% at best.[10,23,35,38]

Previous studies by Franco and colleagues documented that red cells which express HbF (F-cells), whether naturally-occurring or associated with a pharmacologic treatment, undergo selective survival and become enriched by 2-to 3-fold in sickle cell disease, resulting in a progressive increase in total HbF levels over time.[10] These findings would predict that longer administration of HQK-1001 should result in higher levels of HbF than observed in this relatively brief trial in small cohorts.

The complex pathophysiology of sickle cell disease has been increasingly well-characterized, with multiple therapeutic targets identified, yet only one therapeutic to reduce the underlying pathology has been approved in over a decade. Stem cell transplant, the one curative approach, is available for a minority of patients for whom appropriate donors are found.[37] Despite documented benefit from HbF induction >0.5 g/dl with hydroxyurea, many adults still suffer chronic morbidity, and there is still an early mortality rate > 40%.[17-18] Several prior generation short chain fatty acids have HbF-inducing activity, and are appealing for their targeted effects in displacing repressor complexes, including BCL-11A and HDAC-3, and recruiting EKLF, but their feasibility for chronic use is problematic due to pharmacokinetic challenges.[28,32,38-9] These early clinical findings now with sodium 2,2 dimethylbutyrate, in combination with prior studies in molecular, cellular, and nonhuman primate models, strongly suggest that HQK-1001 offers potential for increasing HbF in sickle cell disease without cytotoxicity, and its pharmacokinetic profile is feasible for long-term administration. Basal HbF levels vary widely in association with diverse genetic modifier profiles, which are likely to influence responses to HbF-inducing therapeutics. [40-48] It is therefore encouraging that five of seven subjects who received the highest dose level had a detectable rise in HbF above baseline in the presence of quite different basal HbF levels in this brief time-frame. Further trials with longer administration of HQK-1001 in larger numbers of sickle cell subjects appear warranted to more definitively evaluate its therapeutic potential.

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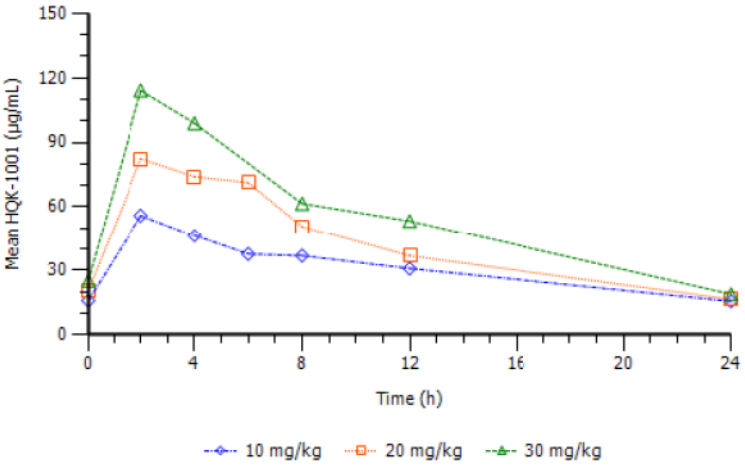

FIGURE 1.
Mean plasma levels of HQK-1001 on day 6 are shown by dose cohort.

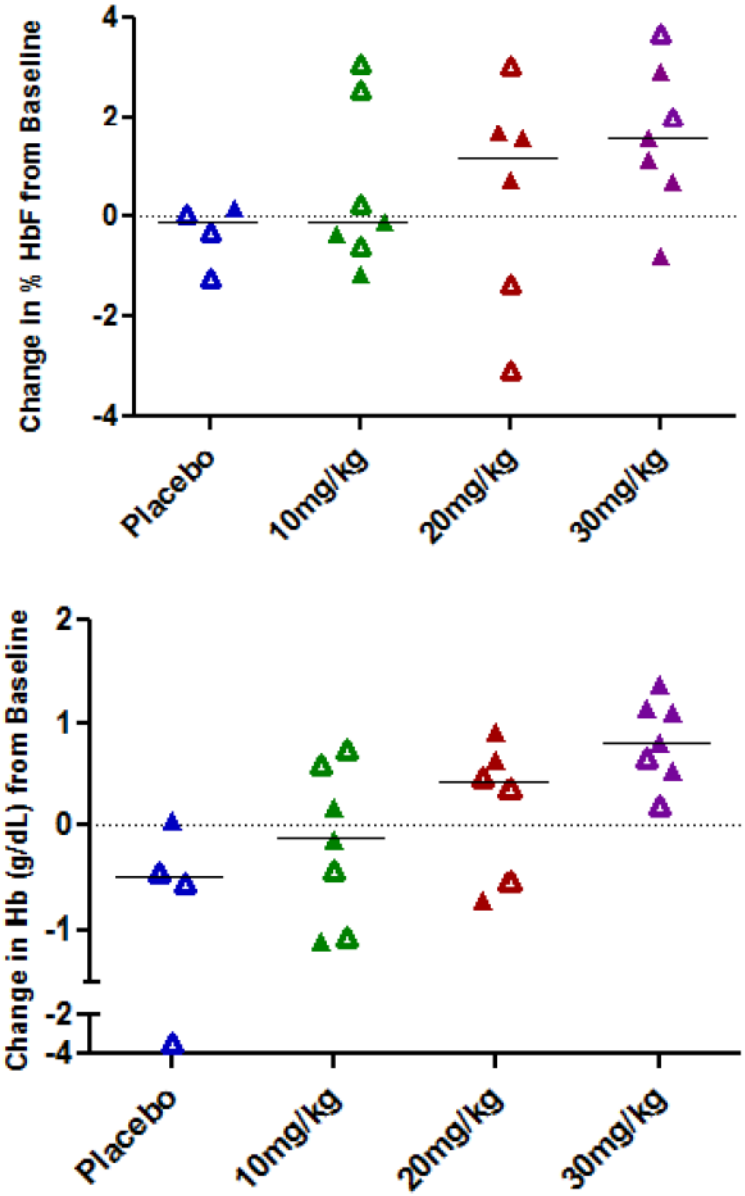

FIGURE 2.

Changes from baseline in % HbF (top panel) and total Hgb (bottom panel) in the 3 dose cohorts.

A. Changes in % HbF are shown in individual subjects in each of the 4 treatment groups. Open symbols represent values in subjects on concomitant hydroxyurea treatment.

B. Changes from baseline in total Hgb are shown in individual subjects in each of the 4 treatment groups. Open symbols represent values in subjects on concomitant hydroxyurea treatment.

Table IA

Basic characteristics

Characteristics	HQK-1001 (n=20)	Placebo (n = 4)
Male Gender	11 (60 %)	3 (75%)
Age in years, median (Range)	29 (13-56)	34 (20-48)
Subjects taking Hydroxyurea	9 (45 %)	3 (75 %)
Weight in kg, median (Range)	63 (53 – 94)	59 (54 – 74)

Table IB

Baseline total hemoglobin and % HbF in individual subjects. Hydroxyurea treatment status is designated in the far right column

ID	Group	Hb (g/dL)	HbF (%)	HU
90301	Placebo	10.0	3.0	Yes
90803	Placebo	8.7	18.3	Yes
91101	Placebo	7.4	2.5	
91103	Placebo	9.9	10.8	Yes
90101	10 mg/kg	9.2	7.7	Yes
90102	10 mg/kg	8.8	5.8	Yes
90103	10 mg/kg	11.9	14.7	Yes
90202	10 mg/kg	9.3	8.4	
90203	10 mg/kg	8.0	2.9	
91102	10 mg/kg	6.1	1.7	
91104	10 mg/kg	5.9	12.2	Yes
90105	20 mg/kg	9.5	32.8	Yes
90204	20 mg/kg	8.5	8.3	
90205	20 mg/kg	9.2	7.0	
90801	20 mg/kg	11.8	10.2	Yes
90802	20 mg/kg	9.9	28.2	Yes
90901	20 mg/kg	11.4	12.2	
90106	30 mg/kg	10.3	13.0	
90206	30 mg/kg	6.3	6.4	
90804	30 mg/kg	9.5	19.8	Yes
90805	30 mg/kg	8.4	2.9	
90806	30 mg/kg	10.2	8.7	Yes
90902	30 mg/kg	7.5	3.7	
91301	30 mg/kg	7.6	22.2	

Table IC

Baseline total hemoglobin and % HbF in individual subjects. Hydroxyurea treatment status is designated in the far right column

ID	Group	Hb (g/dL)	HbF (%)	HU
90301	Placebo	10.0	3.0	Yes
90803	Placebo	8.7	18.3	Yes
91101	Placebo	7.4	2.5	
91103	Placebo	9.9	10.8	Yes
90101	10 mg/kg	9.2	7.7	Yes
90102	10 mg/kg	8.8	5.8	Yes
90103	10 mg/kg	11.9	14.7	Yes
90202	10 mg/kg	9.3	8.4	
90203	10 mg/kg	8.0	2.9	
91102	10 mg/kg	6.1	1.7	
91104	10 mg/kg	5.9	12.2	Yes
90105	20 mg/kg	9.5	32.8	Yes
90204	20 mg/kg	8.5	8.3	
90205	20 mg/kg	9.2	7.0	
90801	20 mg/kg	11.8	10.2	Yes
90802	20 mg/kg	9.9	28.2	Yes
90901	20 mg/kg	11.4	12.2	
90106	30 mg/kg	10.3	13.0	
90206	30 mg/kg	6.3	6.4	
90804	30 mg/kg	9.5	19.8	Yes
90805	30 mg/kg	8.4	2.9	
90806	30 mg/kg	10.2	8.7	Yes
90902	30 mg/kg	7.5	3.7	
91301	30 mg/kg	7.6	22.2	

Table IIA

Adverse events listed as serious

Serious Adverse Events		
SAE Terms	**HQK-1001 (n=20) N (%)**	**Placebo (n=4) N (%)**
Crisis	5 (25)	3 (75)
Anemia	1 (5)	0
Catheter site infection	1 (5)	0
Arthralgia	1 (5)	0
Pain in extremity	1 (5)	0

Table IIB

Most common adverse events, occurring in 10% or more of subjects

Event	HQK-1001 (n = 20, all doses) % of subjects	Placebo (n = 4) % of subjects
Sickle cell crisis	45	75
Nausea	30	25
Pain in extremity	30	0
Cough	30	0
Arthalgia	25	25
Headache	25	25
Chest Pain	15	25
URI	15	25
Back Pain	15	25
Vomiting	15	0