Title:	ROLE OF GLP-1 IN CONGENITAL HYPERINSULINISM: EFFECT OF EXENDIN-(9-39) ON GLUCOSE REQUIREMENTS TO MAINTAIN EUGLYCEMIA
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List of Abbreviations

CHI: congenital hyperinsulinism

Ex-9: Exendin-(9-39)

GLP-1: glucagon-like peptide-1

GLP-1r: glucagon-like peptide-1 receptor

K_{ATP}: ATP- sensitive potassium channel

K_{ATP}HI: congenital hyperinsulinism due to mutation in the K_{ATP} channel

GIR: glucose infusion rate

Study Summary

Title	Role of GLP-1 in Congenital Hyperinsulinism: Effect of exendin-(9-39) on glucose requirements to maintain euglycemia
Short Title	Effect of Ex-9 on glucose requirements
Phase	Phase 1/2
Methodology	Open label study
Study Duration	2 years
Study Center(s)	Single-center
Objectives	To examine the effect of exendin-(9-39) on glucose requirements to maintain euglycemia in subjects with congenital hyperinsulinism unresponsive to medical therapy
Number of Subjects	Approximately 30 subjects
Diagnosis and Main Inclusion Criteria	Congenital hyperinsulinism Main inclusion criteria: unresponsive to medical therapy with diazoxide
Study Product, Dose, Route, Regimen, Duration of administration	Exendin (9-39) 1000-30000 pmol/kg/min (0.2-6 mg/kg/hr) intravenous infusion over 9 hours.
Reference therapy	Effect will be evaluated compared to vehicle (normal saline) baseline
Statistical Methodology	Average glucose infusion rate during the last 2 hours of exendin-(9-39) infusion will be compared to glucose infusion rate during the last 2 hours of vehicle infusion. Standard t-test, $p<0.05$

Schedule of Events

STUDY DAYS 1 & 2*

Procedures	Day 0 (regardless of group assignment)	Vehicle infusion day	Exendin – (9-39) infusion day
Hypersensitivity test[1] chemistry profile,electrocardiogram,CBC,urinalysis[2]	x		
Heart rate, blood pressure, respiratory rate, temperature		x	x
Maintain blood glucose >70mg/dL - dextrose infusion rate will be adjusted to maintain glucose in the range of 70-90mg/dL[3]		x	x
Infuse Saline (vehicle) for 9 hours		x	
Infuse exendin-(9-39) for 9 hours			x
Blood glucose will be checked every 30min by heel stick during study period		x	x
Betahydroxybutyrate will be checked every hour by heel stick during the study period		x	x
Plasma levels of exendin-(9-39) 1hr, 5hr, 9hr during study period and, 1hr, 3hr and after infusion is discontinued			x
Plasma glucose and plasma insulin will be measured at 0,1, 5, and 9 hours		x	x

[1] Test of immediate hypersensitivity may be done the day prior or the morning of the study prior to the start of the infusion.
[2] Chemistry profile, CBC, and urinalysis will be repeated at end of study.
[3] Blood glucose will be checked every 30 minutes by heel stick during study period

*Day 1 and 2 crossover randomized design

Schedule of Blood Drawing

Study Day 1 & 2:
(crossover randomized design)

Total blood volume for Day 1 is 9.4 mL about 2 teaspoons
Total blood volume for Day 2 is 18.2 mL about 3 2/3 teaspoons
Total blood volume for safety laboratories before and after the study is 10 mL

Blood test	Time points				Post infusion	
	0hr	1hr	5hr	9hr	10hr	12hr
Blood Glucose[1]	x	x	x	x	x	x
Betahydroxybutyrate[2]	x	x	x	x	x	x
Plasma glucose and plasma insulin	x	x	x	x		x[3]
Plasma levels of exendin- (9-39) (study drug day only)		x	x	x	x	x

[1] Blood glucose will be checked every 30 minutes by heelstick. Subjects who have discontinued the dextrose infusion at hour 9 during the exendin-(9-39) infusion will continue to have glucose levels checked every 30 minutes up to hour 12 or until blood glucose < 70 mg/dL, whichever comes first
[2] Betahydroxybutyrate will be checked hourly by heel stick. Subjects who have discontinued the dextrose infusion at hour 9 during the exendin-(9-39) infusion will continue to have betahydroxybutyrate checked every hour up to hour 12 or until blood glucose < 70 mg/dL, whichever comes first
[3] Subjects who have discontinued the dextrose infusion at hour 9 during the exendin-(9-39) infusion will have plasma glucose and plasma insulin levels checked either at hour 12 or when blood glucose < 70 mg/dL, whichever comes first

Role of GLP-1 in Congenital Hyperinsulinism

1. Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1. *Background Information and Rationale*

1.1.1. Congenital Hyperinsulinism

In infants and children, as in adults, the most common cause of hypoglycemia is hyperinsulinism (HI). However, unlike adults, HI in children most often represents a genetic disorder of insulin regulation rather than an acquired islet tumor (1). Congenital HI (CHI) is a genetic disorder of pancreatic β-cell function characterized by failure to suppress insulin secretion in the presence of hypoglycemia, resulting in brain damage or death if inadequately treated. The estimated incidence of CHI in this country is 1:20,000 to 1:50,000. Mutations in nine genes have been associated with CHI: *ABCC8*, the gene encoding the sulfonylurea receptor-1 (SUR-1) (2); *KCNJ11*, the gene encoding the inward rectifying potassium channel (Kir6.2) (3; 4); glucokinase (*GCK*) (5); glutamate dehydrogenase (*GLUD-1*) (6); *HADHSC* encoding the mitochondrial enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) (7); and *SCL16A1*, which encodes the monocarboxylate transporter 1 (MCT1) (8); *HNF1A,* the gene encoding hepatocyte nuclear factor 1 alpha (9); and *UCP2*, encoding uncoupling protein 2 (10). Hyperinsulinism can also be associated with Beckwith Wiedemann syndrome, which results from various genetic or epigenetic anomalies in the highly imprinted region of chromosome 11p15.5 (11). Loss-of-function mutations in the K_{ATP} channel (composed of two subunits: Kir6.2 and SUR-1) are responsible for the most common and severe form of HI (K_{ATP}HI), with most patients requiring near-total pancreatectomy to control hypoglycemia, leading to prolonged hospitalization and life threatening complications (1). Overall, inactivating K_{ATP} channel mutations are found in 90% of cases that require surgery. In 9% of these cases a mutation is not identified (12).

The K_{ATP}-sensitive channels couple the metabolic state of the β-cell to membrane potential by responding to changes in intracellular ATP concentration. In pancreatic β-cells, closure of the channel by the elevation of ATP/ADP ratio following stimulation with glucose leads to depolarization of the membrane and activation of a voltage-dependent calcium channel that ultimately results in exocytosis of insulin-containing granules (13). Diazoxide, the mainstay of medical therapy for hyperinsulinism, suppresses insulin by promoting the opening of the β-cell K_{ATP} channels; therefore it is ineffective in patients with K_{ATP}HI. The second line of medical therapy for hyperinsulinism is octreotide, a somatostatin analog.

The initial response to octreotide is good in most infants with hyperinsulinism but tachyphylaxis develops after a few doses, rendering therapy inadequate for long-term use in most cases (14). Calcium channel blockers have also been tried unsuccessfully (15). Thus, most of these patients require surgical palliation by near-total pancreatectomy, which is not curative but carries a high risk of either persistent hypoglycemia or insulin-requiring diabetes (1).

We have now proof-of-concept data in both animal models (16) and in affected patients (17) indicating that exendin-(9-39) is a promising potential therapy for infants with CHI that cannot be treated with currently-available medications (see preliminary results). In an animal model of K_{ATP}Hyperinsulinism (*SUR-1*$^{-/-}$ mouse), a continuous infusion of exendin-(9-39) corrected the fasting hypoglycemia by inhibiting insulin secretion. Furthermore, in islets isolated from these mice, exendin-(9-39) did not only inhibit baseline insulin secretion but also blocked amino acid-stimulated insulin secretion (16), suggesting that this peptide may correct both fasting hypoglycemia and protein-induced hypoglycemia in affected human subjects.

1.1.2. Clinical Phenotype of KATP Hyperinsulinism

K_{ATP}HI is characterized by large for gestational age birth weight (due to stimulation of fetal growth by excessive insulin *in utero*), neonatal onset of hypoglycemia, and unresponsiveness to diazoxide therapy. In addition to fasting hypoglycemia, children with K_{ATP}HI have protein-induced hypoglycemia (18). Most reported K_{ATP} channel mutations are recessive, however a few dominantly expressed mutations have been reported (19-21). The dominant defects retain responsiveness to diazoxide and tend to be milder.

There are at least two distinct histological forms of K_{ATP}HI, diffuse hyperinsulinism and focal hyperinsulinism. In focal hyperinsulinism (approximately 40-60% of all cases) a somatic reduction to homozygosity (or hemizygosity) of a paternally inherited mutation of *ABCC8* or *KCNJ11* and a specific loss of maternal alleles of the imprinted chromosome 11p15 region result in a focal lesion. Pre-operative identification and localization of these focal lesions by ^{18}F-fluoro-L-DOPA facilitates local resection of the lesion and cure of this form of hyperinsulinism (22-24). Infants with diffuse hyperinsulinism require a near-total pancreatectomy (98%) to control the hypoglycemia and often require additional therapy with octreotide, and/or frequent feedings to maintain euglycemia. At The Children's Hospital of Philadelphia's Congenital Hyperinsulinism Center, the largest center in this country caring for children with disorders of insulin regulation, we evaluate and treat approximately 20-30 infants with medically-unresponsive hyperinsulinism requiring pancreatectomy every year. Among cases with diffuse hyperinsulinism, approximately 54% had persistent hypoglycemia after a near-total pancreatectomy, up to 24% required a second pancreatectomy for persistent hypoglycemia, 18% had persistent hypoglycemia but were medically controlled, and 20% had insulin-requiring diabetes. Thirty-one percent of the cases had surgical complications, including bowel obstruction, pancreatic exocrine insufficiency, necrotizing enterocolitis and death (1 from necrotizing enterocolitis and 1 from bowel obstruction).

1.1.3. Metabolism and Insulin Secretion in Islets Lacking KATP Channels

Animal models have been generated to replicate the defect in human K_{ATP}HI. Mice lacking Kir6.2-containing K_{ATP} channels (*Kir6.2*$^{-/-}$ mice) show transient hypoglycemia as neonates. With age the *Kir6.2*$^{-/-}$ mice develop fasting hyperglycemia and glucose intolerance (25). SUR-1 knockout (*SUR1*$^{-/-}$) mice have also been generated (26). *SUR1*$^{-/-}$ mice are both significantly more hyperglycemic when glucose-loaded and significantly more hypoglycemic when fasted than the control animals (27). Despite their impaired response to glucose, *SUR1*$^{-/-}$ mice secrete normal amounts of insulin after a meal, through intact second phase insulin secretion (28).

Isolated islets from *SUR1*$^{-/-}$ mice, exhibit all the features expected to result from nonfunctional K_{ATP} channels, including β-cell depolarization and elevation of intracellular calcium (28; 29). *SUR-1*$^{-/-}$ rodent islets exhibit an abnormal pattern of basal and fuel-stimulated insulin secretion. Basal insulin secretion is two-three fold higher in *SUR-1*$^{-/-}$ islets compared to wild type controls (30; 31). Although the pattern of glucose-stimulated insulin secretion in these islets has been an issue of controversy, in all but one study (32) the response to high glucose was virtually absent or small compared to control islets (28-30; 33; 34). Stimulation by acetylcholine and GLP-1 have been shown to increase glucose responsiveness in these islets (34). In contrast to the poor response to glucose, isolated *SUR-1*$^{-/-}$ islets are hypersensitive to amino acids. After stimulation with an amino acid mixture, insulin release increases by 3-fold in *SUR-1*$^{-/-}$ islets, while normal islets are not affected by this stimulus, unless depolarized with glucose or sulfonylureas (30). These findings are congruent with the clinical observation of protein sensitivity in K_{ATP}HI (35). Evidence from *SUR-1*$^{-/-}$ islest (36) and *Kir6.2*$^{-/-}$ (37) and *SUR-1*$^{-/-}$ (38) mice demonstrating impaired glucagon secretion implicates K_{ATP} channels as regulators of glucagon secretion. The role of K_{ATP} channels in glucagon secretion seems to be independent of insulin and directly regulated by alpha cells K_{ATP} channels (39).

Isolated islets from human subjects with K_{ATP} hyperinsulinism have also been studied but in a more limited way. Electrophysiological studies of islets from infants with HI show an absence of K_{ATP} channel activity and constitutively elevated intracellular calcium. Basal insulin secretion from these islets was 10-fold higher than controls. In static incubations, glucose caused release of insulin in a dose-dependent manner and acetylcholine, ATP and UTP also increased insulin secretion (40).

1.1.4. Glucagon-like Peptide-1 Receptor Agonists and Antagonists

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by enteroendocrine L-cells in response to ingested nutrients. GLP-1 was first reported in 1987 to be a potent stimulator of glucose-dependent insulin release (41). Since then, numerous studies using GLP-1 agonists, antagonists, as well as genetic loss-of and gain-of-function rodent models have demonstrated that GLP-1 not only enhances postprandial insulin secretion (42-44) but has other actions that are complementary to the incretin effect, including inhibition of glucagon

secretion (45; 46), hepatic glucose production (47; 48), gastric emptying (49; 50), and appetite (51; 52).

The major physiological stimulus to L-cells is nutrient ingestion, with circulating levels of GLP-1 rising by two- to threefold in response to a mixed meal in humans (53). The regulation of GLP-1 secretion by ingested nutrients is complex and thought to involve indirect vagal pathways as well as direct luminal stimulation of the intestinal L-cells (54). Studies in a GLP-1-secreting cell line (GLUTag) have demonstrated that glucose and other metabolized sugars enter the L-cells (likely via a glucose transporter, GLUT-1 or GLUT-5) and induce depolarization by closure of K_{ATP} channels (Kir6.2/SUR-1) (55; 56). A rise in cytoplasmic Ca^{2+} concentration due to opening of voltage gated calcium channels is part of the secretion mechanism (57). A different mechanism involving electrogenic substrate entry to the cell through sodium-glucose transporters results in K_{ATP} channel independent glucose-stimulation of GLP-1 release (58). Given the common embryological origin of the pancreas and intestine it is not surprising that the enteroendocrine cells and β-cells share similar mechanisms of glucose sensing and response; furthermore, glucokinase, the "glucose-sensor" in β-cells, is also expressed in these cells (59). GLP-1 secretion has not been studied in K_{ATP}HI, but the observed similarities between pancreatic β-cells and enteroendocrine L-cells suggest that glucose-stimulated GLP-1 secretion may be affected in subjects with K_{ATP}HI. Furthermore, our islet studies suggest that the GLP-1r is constitutively active in *SUR-1*$^{-/-}$ islets (16).

A single GLP-1 receptor (GLP-1r) has been identified and is expressed in the pancreatic islet, the gastrointestinal mucosa, heart, lung, kidney, and the brain (60). A mouse with a targeted gene deletion of the GLP-1r has been shown to have fasting hyperglycemia and glucose intolerance, but no apparent major abnormalities in the function of the lungs, GI tract or CNS (61-63). Studies in cell lines or transfected cells have demonstrated that GLP-1 binds to the GLP-1r and activates intracellular signaling in great part through activation of adenylyl cyclase and the generation of cAMP (64; 65).

The exendin peptides, exendin-3 and exendin-4, were originally isolated from the saliva of the Gila Monster, *Heloderma horridum*, using an assay for N-terminus Histidine residues (66-69). Exendin-3 interacts with the VIP receptor, but exendin-4 is a high affinity agonist of the GLP-1r. Exendin-4 is bound more avidly than GLP-1 (70), and is relatively more potent than GLP-1 in stimulating insulin secretion (71; 72). In addition, because it is not susceptible to metabolism by dipeptidyl-peptidase IV, exendin-4 has a longer circulating half-life than GLP-1 (73; 74). Exendin-4 (Exenatide, Byetta®), is approved for the treatment of type 2 diabetes mellitus, and other GLP-1 analogues are currently in late phase of clinical development (60) . A truncated form of exendin-4, exendin-(9-39), has been shown to be a specific antagonist of the GLP-1r *in vitro* (71; 75) and *in vivo* in a variety of animal models (76-78) and in humans (79). We have shown that antagonism of the GLP-1r by exendin-(9-39) results in a persistent elevation of fasting blood glucose levels in normal mice (80).

In human subjects, Schirra and colleagues used exendin-(9-39) during glucose clamps, with and without concurrent infusions of GLP-1 (79). At a dose of 300 pmol/kg/min (0.06 mg/kg/hr), exendin-(9-39) blocked GLP-1 induced insulin secretion by 85-95%. In addition, administration of exendin-(9-39) increased plasma glucagon concentrations, indicating the importance of GLP-1 in the regulation of the islet α-cell. Edwards et al. also infused exendin-(9-39) into healthy volunteers (81). In this study subjects were given doses of 5-500 pmol/kg/min (0.001-0.1 mg/kg/hr) during infusion of 0.5 pmol/kg/min of GLP-1. Significant antagonism of GLP-1-induced insulin secretion, a 21% decrease, was noted with 50 pmol/kg/min (0.01 mg/kg/hr) of exendin-(9-39), and 500 pmol/kg/min (0.1 mg/kg/hr) completely abolished the effect of exogenous GLP-1. Furthermore, administration of exendin-(9-39) to fasted subjects resulted in increased plasma fasting glucose levels. These investigators also studied the effect of exendin-(9-39) on endogenous GLP-1-stimulation of insulin secretion by infusing 500 pmol/kg/min (0.1 mg/kg/hr) during oral glucose tolerance tests. In this experiment exendin-(9-39) caused glucose intolerance relative to a control infusion of saline. The circulating half-life of exendin-(9-39) was determined to be ~ 30 minutes using a specific radioimmunoassay. Interestingly, in both studies the relative concentrations of exendin-(9-39) necessary to completely block GLP-1 were ~ 1:1000, higher than would have been predicted by the relative binding of these two peptides determined *in vitro* (68; 70; 71). These studies demonstrate that exendin-(9-39) acts as a GLP-1r antagonist in humans, and that GLP-1 has a physiologic role in glucose metabolism in healthy subjects.

1.1.5. ***Significance***

Congenital hyperinsulinism is a devastating disease affecting approximately 100 to 200 neonates each year in the USA. The lack of effective medical therapies results in the need for a near total pancreatectomy to ameliorate the hypoglycemia. This surgical approach carries a high risk of complications such as diabetes, bowel obstruction, malabsorption, necrotizing enterocolitis and death. Furthermore, a significant number of the infants continue to have hypoglycemia after the surgery requiring continuous administration of glucose by a gastrostomy tube to maintain euglycemia. Despite the impressive recent advances in the understanding of the molecular biology of congenital hyperinsulinism, there have not been any advances in terms of medical therapies for over 20 years. As shown in the Preliminary Studies section, we have data showing very promising effects of antagonism of the GLP-1 receptor by exendin-(9-39) not only in a mouse model but also in human subjects with hyperinsulinism due to K_{ATP} channel mutations. The development of this peptide as a therapeutic agent for this disorder would represent a major advance in the field.

1.2. Name and Description of Investigational Product

The principal investigator holds an investigational new drug application for the use of exendin-(9-39) in this population (IND#76,612). This protocol is being added to the original application.

*1.2.1. **Exendin-(9-39)***

Exendin-(9-39), a truncated form of exendin-4 (exenatide, Byetta ®, FDA approved for the treatment of type 2 diabetes mellitus), is a specific antagonist of the GLP-1r with opposite effects on glucose levels than the agonist, exendin-4. In human studies, exendin-(9-39) has been shown to increase fasting plasma glucose levels (81). No side effects, including no effects on blood pressure and heart rate, have been reported with the use of this peptide in normal human subjects (79; 81). Similarly, the peptide has appeared to be well-tolerated in our pilot studies as well (see below). There is limited data on the pharmacokinetics of exendin-(9-39) in human subjects. The circulating half-life of exendin-(9-39) was determined to be ~ 30 minutes using a specific radioimmunoassay (81), but no data on the metabolism of the peptide is available. It is expected that the metabolism of exendin-(9-39) will be similar to the metabolism of the parent peptide, exendin-4 (Exenatide-Byetta®, Amylin Pharmaceuticals, Inc.) from which it differs by only eight amino acids. The intravenous half-life of both peptides is very similar. Non-clinical studies have shown that exenatide is predominantly eliminated by glomerular filtration with subsequent proteolytic degradation. The mean apparent clearance of exenatide in humans is 9.1 L/h and the mean terminal half-life is 2.4 h. These pharmacokinetic characteristics of exenatide are independent of the dose. In patients with mild to moderate renal impairment (creatinine clearance 30 to 80 mL/min), exenatide clearance was only mildly reduced (Product insert, Amylin Pharmaceuticals, Inc.).

*1.2.2. **Chemistry, manufacturing, control***

For our studies, exendin-(9-39) was synthetized by American Peptide Company (Coorporate Offices, 777 East Evelyn Street, Sunnyvale, CA 94086) under cGMP guidelines. The peptide was purified to ≥ 99% by HPLC, and the sequence and mass verified (by Mscan and mass spectral analysis). The investigational drug was formulated by the University of Iowa Pharmaceuticals (115 South Grand Avenue, G-20, Iowa City, Iowa, 52242, a GMP facility) in a 10mM Citrate Buffer, pH 6.0 (Citric Acid Monohydrate USP, Sodium Citrate Dihydrate USP, and d-Mannitol, USP) and stored in sterile 5 mL vials with stopper and seal (West Pharmaceutical) in lyophilized form at -20° C. Each vial contains 25 mg of exendin-(9-39). For administration, the Investigational Pharmacy at the Children's Hospital of Philadelphia will dilute the peptide in 0.9% NaCl and 0.25% human serum albumin to a final concentration of 0.1mg/mL. The 25% human serum albumin will be obtained from Grifols or Octapharma (based on availability).

1.2.3. ***Toxicity***

Exendin-4, the naturally occurring *Heloderma* product, has been used extensively in human studies (83-85). Side effects reported with exendin-4 have been limited, although in higher doses nausea has been reported. Exendin-(9-39) is a synthetic molecule that has also been used in multiple human studies (79; 81; 86-91). Dose ranging studies have been reported by both Schirra and Edwards (79; 81). In neither of the previous studies were adverse effects of exendin-(9-39) reported. In addition, no effects of exendin-(9-39) on blood pressure and heart rate were found on these studies. In both animals and humans studies exendin-(9-39) seems to be relatively short-lived in the circulation. While GLP-1 seems to play an important role in glucose tolerance it is not the only regulatory factor involved, and the glucose tolerance induced by exendin-(9-39) is relatively modest. In our studies, infusion of exendin-(9-39) to mice with a null mutation of the K_{ATP} channel did not significantly worsen the glucose tolerance of these mice, nor affected weight gain (16). We have not observed any serious adverse events on the subjects we have studied under this IND: to date, all doses appear to have been generally safe and well tolerated in all three populations studied. There have been no SAEs, deaths, or withdrawals due to study drug.

Toxicology studies were completed in Sprague Dawley rats (7±1 days of age) and beagle dogs (13 days of age). In 14-day non-GLP studies, three daily subcutaneous (SC) doses of 15, 50, and 150 mg/kg/dose were administered to rats and 5, 15, and 40 mg/kg/dose were administered to dogs. Based on the lack of toxicologically significant findings, doses for GLP 28-day studies were increased to 20, 80, and 300 mg/kg/dose three times daily for rats and 10, 30, and 100 mg/kg/dose three times daily for dogs. Toxicological endpoints evaluated included: clinical observations, body weight, food consumption, clinical pathology, CNS assessment (rats), electrocardiography (dogs), toxicokinetics, and histopathology. The only exendin (9-39)-related observations noted were increased serum glucose in female rats and decreased serum triglycerides in male rats. In the cardiovascular safety pharmacology study, beagle dogs (7 to 8 months old) were administered a single SC dose of vehicle or 5, 20, or 80 mg/kg exendin (9-39) in a 4 x 4 Latin-square design. No toxicologically significant findings were observed. A dose proportional increase in exendin-(9-39) plasma exposure was observed; combined average AUC_{last} on Day 28 in male and female rats at the 300 mg/kg/dose was 249,000 ng•h/mL and at the 100 mg/kg/dose in dogs was 340,500 ng•h/mL. In conclusion, based on the available data, no observed adverse effect levels (NOAELs) were 900 mg/kg/day in rats and at 300 mg/kg/day in dogs. The human equivalent intravenous dose based on body surface area and a bioavailability of 70% are 116 mg/kg/day and 102 mg/kg/day.

1.3. Findings from Pre-Clinical and Clinical Studies (see attachments)

1.3.1. Findings from Pre-Clinical Research

We have conducted experiments that showed a significant effect of exendin-(9-39) on fasting blood glucose levels in *SUR1*$^{-/-}$ mice. Mice carrying a null mutation in the SUR-1 gene exhibit significant fasting hypoglycemia (59.4±1.5 mg/dL vs. 75±1.8 mg/dL, p=0.00000003) and glucose intolerance when compared to wild-type littermates.

To evaluate the effect of GLP-1r antagonism in mice lacking K_{ATP} channels, we treated *SUR1*$^{-/-}$ mice with exendin-(9-39). Twelve-18 wk old male *SUR1*$^{-/-}$ (n=22) and wild type littermates (n=23) were randomized to treatment or vehicle. The treatment group received a continuous infusion of exendin-(9-39) at a dose of 150 pmol/kg/min (0.03 mg/kg/hr) via a subcutaneous mini-osmotic pump (Alzet model 2002) for 2 wks. The control group received an infusion of vehicle (0.9%NaCl/1%BSA). On day 7, fasting blood glucose was significantly lower in vehicle-treated *SUR-1*$^{-/-}$ mice compared to vehicle-treated wild-type littermates (p=0.000002). Treatment with exendin-(9-39) significantly raised fasting blood glucose in *SUR-1*$^{-/-}$ mice compared to vehicle-treated *SUR-1*$^{-/-}$ mice (82.2±6.3 mg/dL vs. 63.2±4.9 mg/dL, p=0.03, on day 3; 82±4.7 mg/dL vs. 56.4±4.3 mg/dL, p=0.0006, on day 7). Fasting insulin/glucose ratio was increased in *SUR-1*$^{-/-}$ mice compared to wild-type littermate control mice (p=0.04) and was normalized by exendin-(9-39) treatment (WT *vs. SUR-1*$^{-/-}$Ex-(9-39): p= 0.32), suggestive of a direct islet effect of exendin-(9-39) on insulin secretion. Insulin sensitivity determined by the measurement of blood glucose in response to an intraperitoneal injection of insulin (0.5 U/kg) was unaffected by treatment. Similarly, glucose tolerance, which is impaired in *SUR-1*$^{-/-}$ mice, was not significantly affected by treatment with exendin-(9-39) (fig. 5). Weight gain was not different in the experimental groups.

The *in vivo* studies were confirmed by perifusion studies. Islets were isolated from *SUR1*$^{-/-}$ mice by collagenase digestion and cultured for 3 days in RPMI 1640 medium containing 10 mM glucose. Batches of 100 cultured islets were loaded onto a nylon filter in a chamber and perifused with Krebs-Ringer bicarbonate buffer with 0.25% bovine serum albumin at a flow rate of 2mL/min. Islets were perifused with a ramp of a physiologic mixture of amino acids (0-12 mM) in the presence or absence of exendin-(9-39) at a concentration of 100 nM. As previously reported (30) the *SUR1*$^{-/-}$ islets abnormally released insulin in response to a mixture of amino acids. This response to amino acids was blocked by exendin-(9-39). The insulin response to KCl was similar in the presence and absence of exendin-(9-39). The effect of exendin-(9-39) on cAMP was determined in static incubations of isolated islets. In the absence of exogenous GLP-1, exendin-(9-39) significantly decreased basal intracellular cAMP in *SUR-1*$^{-/-}$ islets. Amino acids significantly increased cAMP levels in *SUR-1*$^{-/-}$ islets compared to baseline and this effect was significantly reduced by exendin-(9-39). In static incubations, the effect of exendin-(9-39) on cAMP levels mirrored its effect on insulin secretion, suggesting that exendin-(9-39) effects on insulin

secretion in *SUR-1*$^{-/-}$ islets are mediated by changes in cAMP. Baseline insulin secretion was significantly reduced by exendin-(9-39). As seen in the perifusion studies, amino acids significantly increased insulin secretion in *SUR-1*$^{-/-}$ islets and exendin-(9-39) significantly reduced amino acid-stimulated insulin secretion. Finally, we studied the effect of exendin-(9-39) on the characteristically elevated intracellular calcium concentration of *SUR-1*$^{-/-}$ islets (30). Exendin-(9-39) did not affect basal or the amino acid-stimulated rise in calcium.

1.3.2. ***Findings from Clinical Research***

Preliminary Studies: Exendin-(9-39) inhibits insulin secretion and elevates fasting blood glucose levels in subjects with K_{ATP} hyperinsulinism (17).

Subjects (n=9,6F) age 15-47 yrs with congenital hyperinsulinism were admitted to the inpatient unit of the CHOP CTRC. After an overnight fast, subjects received an intravenous infusion of vehicle (0.9%NaCl) for 1 hour followed by an intravenous infusion of exendin-(9-39) at three different doses [100, 300 and 500 pmol/Kg/min (0.02, 0.06 and 0.1 mg/kg/hr)] for 2 hours each or vehicle for 6 hours. All subjects underwent the vehicle and exendin-(9-39) infusions in randomized order and on different days. Blood samples for blood glucose, insulin, C-peptide, glucagon, and intact GLP-1 were obtained at multiple time points during the infusions. Exendin-(9-39) increased fasting blood glucose levels in all subjects compared to vehicle [average fasting blood glucose during study on vehicle *vs.* exendin-(9-39): 84±12.2mg/dL vs. 105±23.2mg/dL $p<0.01$]. Six of the subject had hypoglycemia (blood glucose < 70 mg/dL)during vehicle infusion while none of them had hypoglycemia during exendin-(9-39) infusion. Fasting insulin to glucose ratio was significantly lower during exendin-(9-39) infusion compared to vehicle (0.08±0.03 vs. 0.12±0.04, $p=0.01$) (fig.5). Fasting glucagon and GLP-1 levels were not different during exendin-(9-39) infusion compared to vehicle. Exendin-(9-39) was well tolerated with no side effects.

The preliminary pre-clinical and clinical studies demonstrate that antagonism of the GLP-1r by exendin-(9-39): 1) suppresses insulin secretion and elevates fasting blood glucose levels in *SUR-1*$^{-/-}$ mice; 2) suppresses baseline and amino acid-stimulated insulin secretion in *SUR-1*$^{-/-}$ islets; and 3) suppresses insulin and elevates fasting blood glucose levels in human subjects with K_{ATP}HI. The insulin inhibitory effect seems to be mediated by the effect of exendin-(9-39) on intracellular cAMP levels in pancreatic β-cells. The effects of exendin-(9-39) on *SUR-1*$^{-/-}$ islets in the absence of agonist stimulation suggest that the GLP-1 receptor is constitutively active in these islets. Overall, our findings suggest a potential role for GLP-1 in the pathogenesis of K_{ATP} hyperinsulinism. Clearly, the promising effects of exendin-(9-39) in the mouse model of K_{ATP}HI and in subjects with K_{ATP} hyperinsulinism suggest a therapeutic potential for GLP-1 receptor antagonism in this disorder.

1.4. Dose Rationale and Risk/Benefits

The doses used in previous human studies ranged from 30 to 900 pmol/kg/min (0.006 to 0.18 mg/kg/hr) (79; 81; 86-91). Schirra et al.(79) demonstrated that

under physiological post-prandial plasma levels of GLP-1 (achieved by an intravenous infusion) administration of exendin-(9-39) at 30 and 60 pmol/kg/min (0.006 and 0.012 mg/kg/hr) significantly reduced the glucose infusion rate required to maintain blood glucose at 140 mg/dL in normal subjects, while a dose of 300 pmol/kg/min (0.06 mg/kg/hr) totally abolished the elevated demand for exogenous glucose under the experimental conditions. Furthermore, at euglycemia, infusion of exendin-(9-39) at 300 pmol/kg/min (0.06 mg/kg/hr) significantly raised basal glucose and glucagon. Edwards et al.(81) studied the effect of exendin-(9-39) on fasting plasma glucose in normal adult volunteers. They found that exendin-(9-39) infused at a dose of 500 pmol/kg/min (0.1 mg/kg/hr) increased fasting plasma glucose significantly compared with controls infused with saline. After cessation of exendin-(9-39) infusion, the plasma glucose level in response to exendin-(9-39) dropped to that of the control. In summary, these studies demonstrated that at a dose of 300 pmol/kg/min (0.06 mg/kg/hr), exendin-(9-39), abolishes the effects of physiologic post-prandial plasma levels of GLP-1, and that a higher dose of 500 pmol/kg/min (0.1 mg/kg/hr) increases fasting plasma glucose levels in normal subjects. In these studies, exendin-(9-39) was well tolerated by all subjects and there was not effect on pulse or blood pressure. No adverse effects related to exendin-(9-39) were reported in the previously discussed studies.

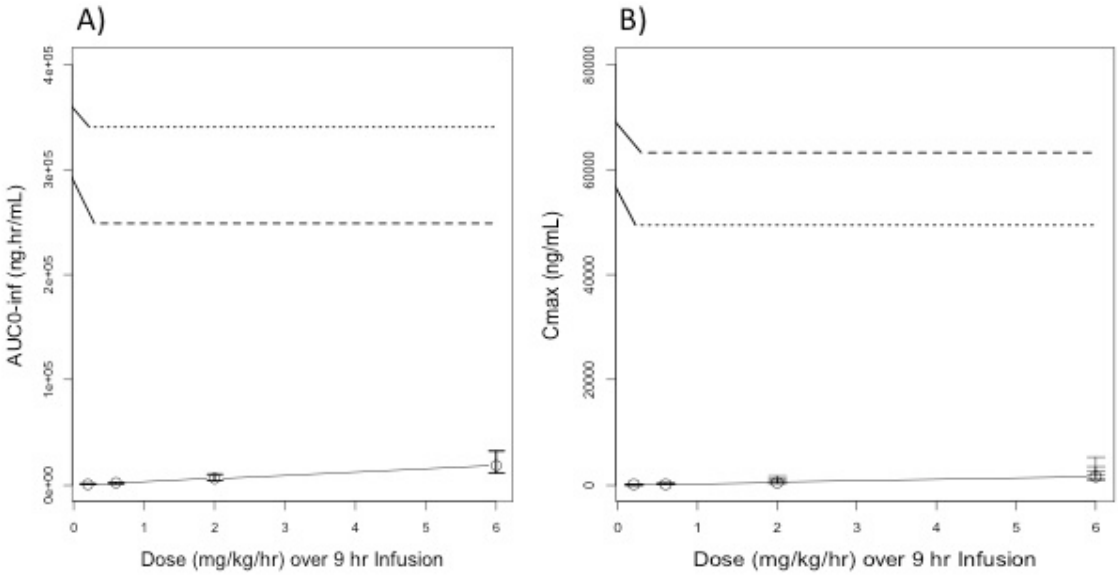
In our studies in mice with a null mutation of SUR-1, a pilot study demonstrated that a dose of 150 pmol/kg/min (0.03 mg/kg/hr) was effective in increasing fasting blood glucose levels, while a lower dose of 50 pmol/kg/min (0.01 mg/kg/hr) did not have an effect. In our pilot human studies, we have used a dose ranging from 100-500 pmol/kg/min (0.02-0.1 mg/kg/hr). From these studies the higher dose (500 pmol/kg/min) was given at the end of the infusion elevating blood glucose levels after a long fast (18 hrs). These doses were well tolerated. We have studied 2 neonates at this dose level administered over 6 hrs and did not find an effect on glucose infusion rates.

We developed a specific and sensitive LC-MS/MS method to quantify the concentration of exendin-(9-39) in human plasma (82) and measured exendin(9-39) concentration serially in individuals who participated in our research studies and received exendin-(9-39) intravenously. A total of 202 PK observations from twenty-six adult and pediatric subjects were included in the analysis. The final parameter estimates were obtained using FOCE with interaction method in NONMEM (version 7.3). A linear two-compartment model best described the observed data, and the PK parameters were estimated with good precision (%CV<50). The PK parameters were related to body weight and age. Gender and creatinine clearance did not affect the clearance of exendin-(9-39) in the studied population. Typical clearance and elimination half-life for pediatric population with median weight 5 Kg are 1.64 L/hr and 0.94 hr, respectively.

The final model parameters are presented in the table below:

PARAMETERS	Population Mean (SE)	Population Variance (%CV)
CL (L/hr)	11.9 (0.781)	0.0722 (0.0309)
VC (L)	8.57 (0.925)	-
Q (L/hr)	3.85 (1.36)	-
Vp (L)	7.00 (1.24)	-
σ_Proportional error	0.310 (0.0381)	-

Based on our clinical data to date including the observed and modeled PK data, we are proposing additional dose-escalation cohorts starting with a dose that is 2-fold higher than the highest dose used in the pilot study [1000 pmol/kg/min (0.2 mg/kg/hr)] up to a maximum dose that is lower than the human equivalent (assuming 70% bioavailability) NOAELs from the toxicology studies in rats and dogs. The estimated range of AUC (A) and C_{Max} (B) with these doses were simulated using the developed population PK model and are shown as follows:



The dotted line is the exposure of the NOELdose in dog. The dashed line is the exposure of the NOEL dose in rat. AUC0-inf = Area under the concentration-time curve from time=0 to infinity; Number of simulated subject = 1000; Open circle = Median; Error Bar = 95 % percentile

Clinical and nonclinical pharmacokinetic parameters and safety factors are summarized in the following table.

Study Type	Dose (mg/kg/hr)	Cmax (ng/mL)	AUC (ng*hr/mL)
Proposed in Neonates	0.2	69.45[a]	625.5[a]
Proposed in Neonates	0.6	208.3[a]	1876[a]
Proposed in Neonates	2	694.5[a]	6255[a]
Proposed in Neonates	6	2083[a]	18764[a]

Rat (Study #13835B)	900 mg/kg/day (300 mg/kg TID)	63200[b]	249,000
Dog (Study #13835D)	300 mg/kg/day (100 mg/kg TID)	49500[c]	340,500

[a] – median value of 1000 simulated subjects; [b] – represented Cmax value of male rats following a single dose administration; [c] – represented Cmax values of female dogs on Day 28

Safety Margins based on dose and exposure (AUC)

Proposed dose rate (mg/kg/hr)	Total dose over 9 hours[a] (mg/kg)	Safety margin based on HED from rat (300 mg/kg/day) [b]	Safety margin based on HED from dog (900 mg/kg/day) [b]	Safety margin based on Cmax from rat (63200 ng/mL)[c]	Safety margin based on Cmax from dog (49500 ng/mL)[d]	Safety margin based on AUC from rat (249000 ng*hr/mL)	Safety margin based on AUC from dog (340500 ng*hr/mL)
0.2	1.8	57	65	910	713	398	544
0.6	5.4	18	22	303	238	133	181
2.0	18	5.6	6.5	91	71	40	54
6.0	54	1.9	2.2	30	24	13	18

[a] – Assuming infusion over nine hours is the daily dose
[b] – For rat HED is 102 mg/kg/day; for dog HED is 117 mg/kg/day; calculations includes bioavailability of 70%
[c] – represented Cmax value of male rats following a single dose administration; [d]– represented Cmax values of female dogs on Day 28

Exendin -(9-39) will be administered intravenously as in the previous human studies. In short-term infusions studies Schirra (79) and Edwards (81) did not find a cumulative or persistent effect once the infusion was discontinued. No adverse effects of exendin-(9-39) were reported in the previously discussed studies. Exendin-(9-39) was well tolerated by all subjects and there was not effect on pulse or blood pressure.

In the children, adolescents and adults with congenital hyperinsulinism that we have studied to date, all doses appear to have been generally safe and well tolerated in all three populations studied. There have been no SAEs, deaths, or withdrawals due to study drug. Adverse events, regardless of causality, were reported in 19 of the 31 subjects (61.3%) treated to date. Treatment-related AEs were reported in only 3 subjects (9.7%). The most common adverse event observed as expected in this population was hypoglycemia (16 of 31 subjects, 51.6%); the majority of these events were Grade 1 or 2 and all were assessed as unrelated to investigational drug. Adverse events possibly related to the investigational drug included emesis (Grade 1) in one subject and transient hyperglycemia in two subjects (Grade 3). The occurrence of adverse events was balanced between the investigational drug and vehicle conditions.

Other than the effects on glucose homeostasis, which appears to be transient in human subjects, we do not anticipate other effects. Therefore, the anticipated benefits derived from these experiments which includes our understanding of the

physiopathologic role of GLP-1 in congenital hyperinsulinism, and moreover, the potential effect of exendin-(9-39) in controlling the severe hypoglycemia affecting these patients in face of the lack of effective medical therapy justify the proposed studies.

1.5. Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonization (ICH). Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report adverse events in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2. Study Objectives

Congenital hyperinsulinism due to mutations in the K_{ATP} channel (K_{ATP}HI) is characterized by severe hypoglycemia unresponsive to available medical therapy. Currently, most patients require a pancreatectomy to control the hypoglycemia, leading to prolonged hospitalization and life-threatening complications. Our pre-clinical studies demonstrate that antagonism of the glucagon-like peptide-1 receptor (GLP-1r) by exendin-(9-39) suppresses insulin secretion and corrects fasting hypoglycemia in mice lacking K_{ATP} channels (*SUR-1*$^{-/-}$ mice). Preliminary results from a clinical pilot study indicate that an intravenous infusion of exendin-(9-39) in older adolescents and adult human subjects with K_{ATP}HI raises fasting blood glucose levels. In this study we will evaluate the effects of exendin-(9-39) on glucose requirements to maintain euglycemia in subjects who have failed medical therapy.

2.1. Specific Aim

To examine the effect of exendin-(9-39) on glucose requirements to maintain euglycemia in infants with congenital hyperinsulinism unresponsive to medical therapy.

Secondary Aim. To determine therapeutic plasma levels, plasma half-life and pharmacokinetics of exendin-(9-39) during an intravenous infusion.

Hypothesis: *In infants with hyperinsulinism unresponsive to medical therapy, antagonism of the GLP-1 receptor by exendin-(9-39) will result in decreased glucose requirements to maintain euglycemia, as a result of suppressed insulin and increased glucagon secretion.*

Rationale: Infants with congenital hyperinsulinism present with severe hypoglycemia shortly after birth. Approximately 50% of these infants are unresponsive to available medical therapy (diazoxide and octreotide) and require a pancreatectomy to ameliorate the hypoglycemia. Before surgery, the maintenance of euglycemia is dependent on high glucose infusion rates (up to 20-25 mg/Kg/min) by intravenous or central lines. This practice is frequently associated with severe complications including sepsis and fluid overload which in the presence of the characteristic myocardial hypertrophy seen in these patients puts them at high risk for heart failure. Based on published studies demonstrating that exendin-(9-39) reduces the glucose infusion rates required to maintain blood glucose levels at a target level in normal human subjects (79) and our preliminary data demonstrating the insulin suppressant effects of exendin-(9-39) in subjects with hyperinsulinism, we propose that by continuously infusing exendin-(9-39) we will be able to reduce the glucose infusion rates needed to maintain euglycemia in infants with congenital hyperinsulinism.

Primary outcome variable: glucose infusion rate during the last 2 hrs of infusion of exendin-(9-39).

Secondary outcome variables: plasma glucose 1 hr after initiation of infusion, insulin, and betahydroxybutyrate levels measured at different intervals during the study. Exendin-(9-39) plasma levels.

Safety variables: Primary safety endpoints will include all types of adverse experiences, in addition to laboratory safety tests (hematology, chemistry, and urinalysis). 12-lead electrocardiograms (ECGs), vital signs and physical examinations will also be recorded**.**

3. Study Design

3.1. General Design

This is an open label randomized, crossover study aim to examine the effects of the investigative peptide, exendin-(9-39) on a key feature of the phenotype of congenital hyperinsulinism: 1) increased glucose requirements to maintain euglycemia.

The proposed human studies will be facilitated by the support of the Clinical and Translational Research Center (CTRC) at The Children's Hospital of Philadelphia and by having access to the largest pool of patients with congenital hyperinsulinism in this country through the Hyperinsulinism Center at The Children's Hospital of Philadelphia.

3.2. Experimental Design

We will compare the glucose infusion rate (measured in mg/Kg/min) required to maintain euglycemia (defined as a blood glucose $\geq$ 70 mg/dL) with a concomitant intravenous infusion of exendin-(9-39) and vehicle in infants with congenital hyperinsulinism unresponsive to medical therapy. Each subject, in random order, will be evaluated on two separate days for a total of 12-15 hours each day.

During the first 3 hours, glucose infusion rates will be titrated to keep blood glucose in the range of 70-90 mg/dL, then, the study treatment period will start. On one day with an infusion of vehicle (normal saline) rates of glucose infusion to maintain blood glucose in the 70-90 mg/dL range will be calculated. To help inform the pharmacokinetic-pharmacodynamic relationship, subjects who have been able to discontinue the dextrose infusion and who are not on supplemental dextrose infusion at the end of the study will continue to be monitored without additional dextrose infusion for up to 3 hours after the end of the study drug infusion, or until blood glucose is < 70 mg/dL (whichever comes first). On the other day, subjects will receive a continuous intravenous infusion of exendin-(9-39) and the glucose infusion rates to maintain blood glucose in the 70-90 mg/dL range will be calculated. Studies will be carried out in the neonatal intensive care unit or the inpatient CTRC unit at The Children's Hospital of Philadelphia.

3.3. Study Population

Subjects will be recruited from patients referred to the Hyperinsulinism Center at The Children's Hospital of Philadelphia, the largest center in the USA, with approximately 30 referrals/year of children with medically-unresponsive hyperinsulinism and an equal number of children with medically-responsive hyperinsulinism. Three hundred and sixty one pancreatectomies have been performed in children with congenital hyperinsulinism within the last 16 years at this center. We plan to enroll approximately 20 additional subjects, beyond the 9 subjects already enrolled up through Amendment 6, making a total number of approximately 30 subjects for about 27 evaluable subjects and to include at least some subjects with diffuse disease.

3.3.1. Inclusion and Exclusion Criteria

Inclusion Criteria:

- Confirmed diagnosis of congenital hyperinsulinism (based on clinical criteria: insulin, beta hydroxybutyrate, and/or free fatty acid plasma levels at the time of hypoglycemia, and/or glycemic response to glucagon at the time of hypoglycemia)
- Age: from birth to 12 months
- Failure to respond to diazoxide (defined as the failure to maintain blood glucose ≥ 70 mg/dL without supraphysiologic rates of glucose infusion:> 4-5 mg/Kg/min)

Exclusion Criteria:

- Evidence of a medical condition that might alter results, including active infection, kidney failure, severe liver dysfunction, severe respiratory or cardiac failure
- Current therapy at the time of initiation of study procedures with medications that affect glucose metabolism, such as high dose glucocorticoids, ß-agonists, glucagon, diazoxide and octreotide. Subjects will be eligible to participate 4 hrs after glucagon discontinuation, 24 hrs after the last dose of octreotide and 72 hours after last dose of diazoxide

- Subjects with suspected Beckwith-Wiedemann syndrome or other syndromic forms of congenital hyperinsulinism

3.4. Study Procedures

1. Test of immediate hypersensitivity. All subjects will have 5 ng of exendin-(9-39) (0.05µg/mL) administered intradermally as a test of immediate hypersensitivity prior to the study. The test of immediate hypersensitivity may be done the day prior or the morning of the study prior to the start of the infusion. A wheal and flare reaction in the 30 minutes following peptide injection will be considered a positive test and the study will be canceled.
2. Baseline liver and kidney function: Baseline laboratories for evaluation of liver and kidney function (ALT, AST, GGT, BUN, creatinine) will be obtained if the subject has not had these tests done within 72 hrs of the study.
3. Safety evaluations: heart rate, blood pressure, respiratory rate and temperature will be measured each day at baseline. Heart rate, blood pressure and respiratory rate will be monitored then hourly during the treatment periods. Temperature will be measured at baseline, hourly during the study, and at the conclusion of the infusion periods. The 12-lead ECG, as well as laboratory safety evaluations including CBC, urinalysis, liver and kidney function will be repeated at the end of the study. Any clinically significant abnormality would be repeated until values have returned to normal range/baseline.
4. Study run-in period: During the 3 hours of run-in period, blood glucose will be monitored every 30 min by heel stick using a bedside glucose meter (Nova StatStrip point-of-care glucose monitor, Nova Biomedical Corporation, Waltham, MA, USA). The dextrose infusion rate will be adjusted to maintain glucose in the range of 70-90 mg/dL. The study run-in period will be timed to start with a feeding in the morning and we will kept consistent between study days.
5. Blood glucose monitoring during the study and maintenance of euglycemia. Blood glucose will be monitored every 30 minutes during the study period by heel stick using a bedside glucose meter (Nova StatStrip point-of-care glucose monitor, Nova Biomedical Corporation, Waltham, MA, USA). Rate of intravenous dextrose infusion will be adjusted as needed to maintain target blood glucose range of 70-90 mg/dL as per standard of clinical practice. If blood glucose concentration is $\leq$ 70 mg/dL a second sample will be obtained immediately for confirmation. Correction of values below the target range will be confirmed by measuring a blood glucose 15 minutes after adjustments to the glucose infusion rate.
6. Feedings: Infants will be allowed to continue feedings by mouth or by nasogastric/gastrostomy tube. Feedings will be continued every 3 hours and the volumes offered will be maintained equal during the two 9 hr study

periods. The volume taken with each feeding will be recorded. Only intravenous glucose will be adjusted to maintain euglycemia.

7. Randomization: The investigational drug services will assign the order of the studies by randomization. They will inform the PI, study coordinator, and CTRC.
8. Intravenous infusion of exendin-(9-39) and vehicle. All subjects will receive an infusion of exendin-(9-39) and vehicle for 9 hours on two separate days and in random order. Exendin-(9-39) /vehicle will be infused intravenously over a period of 9 hours starting at approximately the same time ± 90 minutes on each day. The starting dose of exendin-(9-39) will be 1000 pmol/kg/min (0.2 mg/kg/hr). The dose will be escalated as outlined below. The volume of saline to be infused will be calculated to match the volume rate of exendin-(9-39).
9. Post-infusion follow-up for subjects who have been able to discontinue dextrose infusion. Subjects who have been able to discontinue the dextrose infusion and who are not on supplemental dextrose infusion at the end of the study will continue to be monitored without additional dextrose infusion for up to 3 hours after the end of the study drug infusion, or until blood glucose is < 70 mg/dL (whichever comes first). In addition to blood glucose monitoring every 30 minutes and betahydroxybutyrate every 1 hr from a capillary sample, a venous sample will be drawn at the end of 3 hours (or when blood glucose < 70 mg/dL, whichever comes first) for plasma insulin and glucose.
10. Dose schemes.

    Scheme #1 (completed as of date of Protocol Amendment 7): exendin-(9-39) infused for 12 hrs at a dose of 100 pmol/kg/min (0.02 mg/kg/hr).

    Scheme #2 (completed as of date of Protocol Amendment 7): exendin-(9-39) infused for 12 hrs at a dose of 200 pmol/kg/min (0.04 mg/kg/hr).

    Scheme #3 (completed as of date of Protocol Amendment 7): exendin-(9-39) infused for 6 hrs at a dose of 500 pmol/kg/min (0.1 mg/kg/hr).

    Scheme #4 (new as of date of Protocol Amendment 7): Dose escalation will be done as described below:

    a. Cohorts 1-2: at least 3 and up to 6 subjects per cohort, will be included in each of the first two cohorts. In any of the first 2 cohorts, if discontinuation of intravenous dextrose is achieved in 2 out of the 3 subjects up to three additional patients (at least 2 with diffuse disease, as defined by PET scan and/or genetics) may be enrolled in order to meet the Success Stopping Criteria (see 11a below).
    b. Cohorts 3- 4: four subjects, with at least 2 subjects with diffuse disease. If discontinuation of intravenous dextrose is achieved in 1 or 2 of the 4 subjects, an additional subject with diffuse may be enrolled in order to meet the Success Stopping Criteria (see 11a below).

c. The dose will be increased in ½ log increments between cohorts as specified below:

Group	Dose Scheme (mg/kg/hr for 9 hours)	Total Dose (mg/kg)	Number of Subjects
1	0.2	1.8	3-6
2	0.6	5.4	3-6
3	2	18	3-5
4	6	54	4-6

11. Stopping Criteria:
    a. Success Stopping Criteria: the dose escalation will be stopped if in a cohort at least 2 subjects with diffuse disease are able to have dextrose infusion discontinued for the final 2 hours of the study period.
    b. Safety Stopping Criteria: if an exaggerated pharmacology effect is observed (blood glucose greater than 200 mg/dL for 2 hours off dextrose infusion) dose escalation will be stopped and the dosing algorithm will be modified to proceed with a dose that is 2 fold higher the previous level observed to be safe. Dosing will be stopped if any severe adverse event is observed.
12. Calculation of glucose infusion rate. Glucose infusion rate over the last 2 hours of the treatment period will be calculated by adding the total amount of intravenous glucose (in mg) received over 2 hours divided by the weight in Kg and by time (120 min).
13. Determination of therapeutic plasma levels, plasma half-life and pharmacokinetics of exendin-(9-39). During the infusion with exendin-(9-39) blood samples will be obtained at 1, 5, and 9 hours, and at 1 and 3 hours after the infusion ends. Should there be difficulty in establishing blood drawing lines to obtain samples of the secondary outcome variables the study will proceed with collection of the primary outcome variable only (glucose infusion rate). Plasma levels of exendin-(9-39) will be determined by mass spectometry.
14. Determination of plasma insulin, blood glucose, and betahydroxybutyrate levels: Venous samples for determination of plasma insulin and plasma glucose will be obtained at 0, 1, 5 and 9 hours during the study period. Betahydroxybutyrate will be obtained hourly by heel stick during the study period. For subjects in whom the dextrose infusion has been discontinued by the end of the study infusion betahydroxybutyrate will be obtained every hour until 3 hours post-dose (or when blood glucose $\leq$ 70 mg/dL, whichever comes first). In these subjects an additional sample will be obtained at the end of 3 hours (or when blood glucose < 70 mg/dL, whichever comes first) for plasma glucose and insulin. Should there be

difficulty in establishing peripheral blood drawing lines to obtain samples of the secondary outcome variables, (Insulin, exendin-(9-39) levels) the study will proceed with collection of the primary outcome variable only (glucose infusion rate). Capillary and venous blood glucose will be measured using the Nova StatStrip point-of-care glucose monitor (Nova Biomedical Corporation, Waltham, MA, USA). Betahydroxybutyrate will be measured at the bedside using a ketone meter (PrecisionXtra, Abbott Laboratories). Blood samples for plasma glucose, insulin and betahydroxybutyrate levels will be sent to the hospital chemistry laboratory for processing.

3.5. Sample Size and Data Analysis.

The goal of this study is to find the intravenous dose of exendin-(9-39) (and the plasma concentration) that is necessary to completely abolish the need for exogenous glucose administration to maintain normal blood glucose in children with congenital hyperinsulinism. The sample size of three for each dosing regimen is a sample of convenience and not based on statistical considerations. However, we offer the following considerations based on our previous studies: for the first 9 subjects studied under this protocol the mean ± standard deviation (SD) baseline glucose infusion rate (GIR) was 13.2 ± 4.8 mg/kg/min. Our hypothesis is that exendin-(9-39) will decrease the glucose infusion rate required to maintain blood glucose > 70 mg/dL. A paired test is called for to compare the two conditions – exendin-(9-39) and vehicle; however, since we do not actually know the SD of differences, our sample size calculation is based on the more conservative t-test for independent samples, and we use the SD of 4.8 as an estimate of the common SD; using a two group t-test with a 0.05 two-sided significance level.

Reduction in GIR	**Sample size for 80% power**	**Sample size for 90% power**
25%	34	45
50%	9	12
100%	3	4

3.5.1. Data Analysis

The pharmacometric and statistical analyses of the data obtained from this study will be conducted by the investigators and study statistician.

3.5.2. Variables and time points

Pharmacodynamics (Primary Variable): Glucose infusion rate during the last 2 hours (hours 7-9) of infusion of exendin-(9-39) *versus* vehicle is the primary pharmacodynamic variable of interest, although blood glucose measurements over time will be utilized in exploratory PK/PD analyses.

Pharmacokinetics (Primary Variables): The pharmacokinetic variables of interest include $AUC_{0-\infty}$, AUC_{0-t}, maximal concentration (C_{max}), time to maximal

concentration (T_{max}), concentration at end of infusion (C_{eoi}), steady state volume of distribution (V_{ss}), clearance (CL) and half-life ($t_{1/2}$) of exendin-(9-39). These will be derived through both non-compartmental and model-based methods.

Safety (Secondary Variables): Primary safety endpoints will include all types of adverse experiences, in addition to laboratory safety tests (hematology, chemistry, and urinalysis). 12-lead electrocardiograms (ECGs), vital signs and physical examinations will also be recorded.

Secondary Variables: Plasma insulin, blood glucose and betahydrodybutyrate levels.

3.5.3. Approach to analysis

The following populations are defined for the analysis and reporting of data.

All Subjects as Treated (AST): All subjects who received any dose of the investigational drug. This population will be used for assessment of safety and tolerability.

Per-Protocol (PP): All subjects who received protocol procedures sufficiently to ensure that these data will be likely to exhibit the effects of treatment, according to the underlying scientific model.

3.5.4. Statistical Methods

Analysis Overview: Each subject will undergo two experiments (i.e., either infusion of exendin-(9-39) or infusion of vehicle in random order. A two-treatment, two-period crossover design will be employed, with two sequence groups (i.e., vehicle infusion first followed by exendin-(9-39) infusion *vs.* exendin-(9-39) infusion first followed by vehicle infusion). Subjects will be randomly assigned to one or the other of the two sequence groups. The primary aim of the study is to evaluate the effect of exendin-(9-39) vs. vehicle on glucose infusion rate in subjects with congenital hyperinsulinism, and to measure PK parameters for exendin-(9-39), and PK/PD relationships between glucose infusion rate, blood glucose and exendin-(9-39) exposure. Descriptive statistics will be used to characterize demographic, baseline, and outcome measures in subjects. Means, SDs, 95% confidence intervals (CIs), medians, and minimum and maximum values will be tabulated and reported for all continuous variables, by infusion condition. Histograms will be used to determine extent of skewness, and transformations (e.g. logarithmic) will be considered for seriously skewed data. Frequency counts and percentages will be used for categorical variables (e.g., gender, race, adverse events).

Pharmacodynamics: To assess the effect of exendin-(9-39) on glucose infusion rate, glucose infusion rate (GIR) over the last 2 hours of the treatment period will be calculated by adding the total amount of intravenous glucose (in mg) received over 2 hours divided by the weight in Kg and by time (120 min) during infusion of vehicle and exendin-(9-39). Comparison between the two infusions of GIR will be made using the linear mixed effects models. Similarly, the plasma concentration of insulin, glucose and betahydroxybutyrate will be analyzed as above. Each of the outcome parameters will be analyzed separately using linear mixed effects

models (92), which are ideally suited for the cross-over design, as this design includes both fixed effects (treatment, period, carry-over) and random effects (the subjects). Moreover, software for linear mixed models (e.g., SAS proc MIXED) allows flexible modeling of the covariance structure, deals with unbalanced or missing data, and allows use of covariates that change both at the within and among-individual level.

In the event that certain of the outcomes are not suitable for linear mixed effects modeling, then a series of three-sample t-tests (or Mann-Whitney tests) will be used. The first test will analyze the equality of the carry-over effects by applying the test to the subject totals. If the carry-over effects are statistically non-significant, we will proceed to the second test, which will determine the effect of time period by applying the test to the time period differences. Finally, provided that there are no statistically significant carry-over or time period effects, we will proceed to the third test, analyzing the treatment effects by applying the test to the cross-over differences.

Dose-response and concentration-effect relationships will be examined between blood glucose and exendin-(9-39), as well as between safety endpoints and exendin-(9-39).

Pharmacokinetics: Exendin-(9-39) plasma concentration-time data will be analyzed using a moment-based, non-compartmental approach (NCA) with WinNonlin (version 5.1, Pharsight Corporation). Basic PK metrics including C_{max}, C_{eoi}, T_{max}, and $AUC_{0-\infty}$, and AUC_{0-t} will be calculated in addition to derived parameters such as elimination half-life. A model-based approach will be utilized in parallel to describe the individual pharmacokinetic parameters including exendin-(9-39) clearance (CL) and volume of distribution (VD) once the appropriate structural model (e.g., one or two-compartment) has been determined. Sources of variability will be described and the contribution of patient-specific covariates to explain sources of variation will be examined via a rigorous covariate analysis using nonlinear mixed effect modeling and the NONMEM (version 6) algorithm. Briefly, covariates will be included in the model if deemed significant ($p<0.05$) observed as a reduction in the objective function value (OFV) greater than 3.84 ($\chi 2$ distribution, df=1). Subsequently, covariates will be retained in the model if fixing their value to zero results in greater than 10.84 ($\chi 2$ distribution, df=1) increase in the OFV, at a higher level of stringency ($p<0.001$). Ultimately, the final model will be selected using a combination of goodness-of-fit criteria including diagnostic scatter plots, convergence with at least 3 significant digits, reasonable parameter estimates and precision of parameter estimates. Diagnostic plots of the base model will evaluate the correlation between PK parameters (clearance, volume) with indices of body size (e.g., bodyweight) and, if so, an allometric model will be considered during model development. The underlying basis for this type of model has been previously described as useful in pediatrics in that it incorporates known physiologic relationships in the covariate-parameter model (93). The effects of categorical covariates will be modeled using normalized power model.

Safety: Summary statistics and plots will be generated for the change from baseline values in the vital signs, ECG parameters, and selected laboratory safety parameters, as deemed clinically appropriate. Depending on the safety parameter,

the difference from baseline will either be computed on the original scale (raw change from baseline) or on the log scale and back-transformed for reporting (percent change from baseline). Summary statistics for the raw laboratory safety tests, ECGs, and/or vital signs may also be computed, as deemed clinically appropriate.

4. Subject Selection and Withdrawal

4.1. Inclusion and Exclusion Criteria

Inclusion Criteria:

- Confirmed diagnosis of congenital hyperinsulinism (based on clinical criteria: insulin, beta hydroxybutyrate, and/or free fatty acid plasma levels at the time of hypoglycemia, and/or glycemic response to glucagon at the time of hypoglycemia)
- Age: from birth to 12 months
- Failure to respond to diazoxide (defined as the failure to maintain blood glucose $\geq$ 70 mg/dL without supraphysiologic rates of glucose infusion:> 4-5 mg/Kg/min)

Exclusion Criteria:

- Evidence of a medical condition that might alter results, including active infection, kidney failure, severe liver dysfunction, severe respiratory or cardiac failure
- Current therapy at the time of initiation of study procedures with medications that affect glucose metabolism, such as high dose glucocorticoids, ß-agonists, glucagon, diazoxide and octreotide. Subjects will be eligible to participate 4 hours after glucagon discontinuation, 24 hours after the last dose of octreotide and 72 hours after last dose of diazoxide
- Subjects with suspected Beckwith-Wiedemann syndrome or other syndromic forms of congenital hyperinsulinism

4.2. Rationale for Using Neonates and Children

Congenital hyperinsulinism is a genetic disorder of insulin regulation, most commonly due to mutations in the K_{ATP} channel. Affected individuals present shortly after birth with severe hypoglycemia and require high glucose infusion rates through a central line to maintain safe blood glucose levels, as they are unresponsive to available medical therapy. The majority of these children require a near-total pancreatectomy to ameliorate the hypoglycemia. Approximately 50% of these children will require some medical intervention (frequent or continuous enteral feedings and/or octreotide) after surgery. The severity of the disorder seems to ameliorate with age, as many of the affected adult individuals who did not undergo a pancreatectomy as infants followed by our team have a safe fasting tolerance. Therefore, it is important to perform these studies in the

population more severely affected: infants and children. Studying the effect of the GLP-1 receptor antagonist, exendin-(9-39), on glucose metabolism of these subject population will provide information that can be used to improve their management and in the developing of new therapies.

4.3. Subject Recruitment

Subjects will be recruited from the Hyperinsulinism Center at The Children's Hospital of Philadelphia, the largest center in this country caring for children with disorders of insulin regulation. Every year our center treats approximately 30 children with medically unresponsive hyperinsulinism that require pancreatectomy. Currently we have approximately 1200 in-patient days a year. This put us in a unique position to conduct research directed to develop new therapies for this disorder.

The study will be advertised in the center's website. Additional study information will be posted to the Pediatric Endocrine Society (PES) website. This web page lists clinical research studies that are recruiting pediatric endocrine subjects across the United States and Canada. The purpose of the web page is to inform the pediatric endocrinology community about clinical research studies, and to allow providers and patients/families to identify clinical research studies that may be of interest. Access to the page is unrestricted.

4.4. Early Withdrawal of Subjects

4.4.1. When and How to Withdraw Subjects

Subjects will be withdrawn from the study if the test of hypersensitivity is positive upon admission before the initiation of the study protocol. Subjects will be withdrawn at any time during the study if an unexpected severe adverse event occurs. There are not expected safety concerns for abrupt termination of the study protocol.

4.4.2. Data Collection and Follow-up for Withdrawn Subjects

In the event of withdrawal before completion of the study protocol, data obtained until the time of withdrawal will be collected and used in the final analysis.

5. Safety and Adverse Events

5.1. Definitions

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or

injuries will be regarded as adverse events. Abnormal results of diagnostic procedures will be considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events will be classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined by the half-life of the drug and will be 24 hrs after the end of infusion.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to

report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the IRB, CTRC, and the FDA (per the IND) of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. These agencies will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A laboratory test abnormality considered clinically relevant by the investigator (e.g. the abnormality suggests a disease and/or organ toxicity, or is of a degree that requires active management such as change of dose, discontinuation of the drug, more frequent follow-up assessments, or further diagnostic investigation) should be documented as an adverse event.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery will be reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery will ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

5.2. Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, grouped under one diagnosis when appropriate.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

*Adverse events will be reported to The Children's Hospital of Philadelphia IRB (215-590-2830) and CTRC (215-590-2215), and the FDA (in writing only) annually at the time of continuing review.

Any action resulting in a temporary or permanent suspension of this study (e.g. FDA actions, IRB actions, or actions by a commercial sponsor or by the investigators or co-investigators) will be reported to BioMarin, Pharmaceutical Inc.

5.3. Reporting of Serious Adverse Events

5.3.1. Study Sponsor Notification by Investigator

An adverse event that is serious, related and unexpected, will be reported to the agencies cited previously by telephone or MEMO within 24 hours of the event. A Serious Adverse Event (SAE) form will be completed by the sponsor-investigator and faxed to the above agencies/regulatory bodies, according to current reporting requirements. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to:

The Children's Hospital of Philadelphia IRB (215-590-2830)
The Children's Hospital of Philadelphia CTRC (215-590-2215)
FDA (in writing only)

At the time of the initial report, the following information will be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the investigator will provide further information on the serious adverse event in the form of a written narrative. This will include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events will be provided promptly to the agencies cited.

5.4. Dose Escalation Decisions

Treatment cohorts will be dosed in escalating order as outlined above (section 3.4). Dose escalation decisions will be made by the investigator only after the safety of the previous dose level has been established. Subsequent cohorts will not be dose until safety data are obtained from all subjects in the previous cohort. Safety will be evaluated based on the incidence and severity of adverse events (AEs), clinical laboratory test results, ECGs, and other relevant clinical findings (e.g., heart rate, blood pressure, respiratory rate and temperature). Decisions to dose escalate or halt dose escalation will be based on review of these data by the investigator, the Safety Officer and the pharmacologist.

5.5. Stopping Rules

Subject Specific Stopping Rules

All subjects will be monitored during administration of the investigational product and if any of the events below occur the subject will be discontinued from treatment and followed clinically.

1. Subjects who have a wheal and flare reaction in the 30 minutes following peptide injection for the Hypersensitivity Test will be withdrawn from further investigation due to a positive hypersensitivity test and any further research procedures will be canceled.
2. **Peptide stopping rules:** Should any of the following events occur during administration of the investigational agent, in the setting of a serious adverse event or adverse event that is possibly related to exendin-(9-39), the subject's treatment will be discontinued and the subject will be followed clinically.
    i. Blood pressure instability
    ii. Pulse instability
    iii. Fever

Trial stopping rules:

If 2 subjects experience serious adverse events thought to be probably or definitely related to study procedures or medications we will place the study on a voluntary hold to evaluate safety and study design. The IRB, FDA, and the Safety Officer will be informed and consulted as appropriate and per local and federal regulations.

Potential study related SAEs include the following:

1. Anemia requiring hospitalization as a consequence of the amount of blood being collected in study procedures
2. Severe hypersensitivity reaction requiring hospitalization

Surveillance of subjects will be performed to permit early detection of adverse events as discussed in the Data and Safety Monitoring Plan.

5.6. Medical Monitoring

It will be the responsibility of the Principal Investigator to oversee the safety of the study at this site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

5.6.1. Data and Safety Monitoring Board

Given the nature of the study a Data and Safety Monitoring Board (DSMB) is not necessary. The attached data safety and monitoring plan include the establishment of a Safety Officer. The safety of the subjects enrolled on the study will be assessed by the Safety Officer. All SAE 's will be reviewed as they occur. Reporting requirements to the IND will be strictly adhered to. If more than one unexpected or previously undescribed serious adverse event attributable to exendin-(9-39) is observed, accrual to the protocol will be suspended. The Safety Officer will assess the risk to the subject, and a recommendation to continue with the study or close it will be made to the IRB for review. Should the decision be made to continue with the study, the modifications to the protocol, the updated assessment of risks and benefits, and a modified informed consent will be submitted to the IRB and FDA for consideration.

6. Data Handling and Record Keeping

6.1. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

6.2. Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

6.3. Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, we will draw a single straight line through the incorrect entry and enter the correct data above it. All such changes will be initialed and dated. WE WILL NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, we will print the clarification above the item, then initial and date it.

6.4. Records Retention

It will be the investigator's responsibility to retain study essential documents for at least 2 years after study completion. These documents will be retained for a longer period if required by an agreement with the agencies cited above. In such an instance, it will be the responsibility of the agency to inform the investigator as to when these documents no longer need to be retained.

7. Study Monitoring, Auditing, and Inspecting

7.1. Study Monitoring Plan

This study will be monitored according to the monitoring plan in Attachment. The investigator will allocate adequate time for such monitoring activities.

7.2. Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, CTRC, Biomarin, and government regulatory bodies (FDA) of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

8. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator will provide a list of IRB members and their affiliate if requested.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form will be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

9. Study Finances

9.1. Funding Source

This study will be financed, in part, through a grant from BioMarin Pharmaceutical Inc.

9.2. Conflict of Interest

All investigators will follow the Children's Hospital of Philadelphia conflict of interest policy.

9.3. Subject Stipends or Payments

Subjects will not be reimbursed for participation in this study.

10. Publication Plan

The investigators hold the primary responsibility for publication of the any results of the study.

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