EPOETIN HOSPIRA

A PROPOSED BIOSIMILAR TO EPOGEN/PROCRIT (EPOETIN ALFA)

BRIEFING DOCUMENT

FOR THE ONCOLOGIC DRUGS ADVISORY COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AESI	Adverse Event Of Special Interest
ADA	Anti-Drug Antibody
ANCOVA	Analysis Of Covariance
anti-rhEPO	Anti-Recombinant Human Erythropoietin
Asn	Asparagine
Asp	Aspartic Acid
AUC	Area Under The Serum Concentration-Time Curve
AUC_{0-48}	Area Under The Serum Concentration-Time Curve From Time 0 To 48 Hours Post-Dose
AUC_{0-t}	Area Under The Concentration-Time Curve From Time Zero To Time Of Last Measurable Concentration
AUEC	Area Under The Effect-Time Curve
$AUEC_{0-t}$	Area Under The Effect-Time Curve From Time Zero To Time Of Last Measurable Reticulocyte Count
$AUEC_{Hb}$	Area Under The Effect Curve For Hemoglobin
BAEC	Baseline-Adjusted Epoetin Concentration
BFU-E	Burst-Forming Unit-Erythroid
BLA	Biologics License Application
BMI	Body Mass Index
BPCI	Biologics Price Competition And Innovation
ºC	Degree Celsius
CFU-E	Colony-Forming Unit-Erythroid
CHO	Chinese Hamster Ovary
CI	Confidence Interval
CIA	Chemotherapy-Induced Anemia
CKD	Chronic Kidney Disease
C_{max}	Maximum Observed Concentration
CQA	Critical Quality Attribute
CRP	C-Reactive Protein
Cys	Cysteine
CZE	Capillary Zone Electrophoresis
dL	Deciliter
DP	Drug Product
DS	Drug Substance
DSC	Differential Scanning Calorimetry
ECG	Electrocardiogram
EMA	European Medicines Agency

Abbreviation	Description
E_{max}	Maximum Observed (Pharmacodynamic) Effect
EPO-R	Erythropoietin Receptor
ESA	Erythropoiesis-Stimulating Agent
Far-UV CD	Far Ultraviolet-Circular Dichroism
FAS	Full Analysis Set (Population)
FDA	Food And Drug Administation
g	Gram
Gal	Galactose
GlcNAc	N-acetylglucosamine
Gln	Glutamine
GLP	Good Laboratory Practices
GMR	Geometric Mean Ratio
Hb	Hemoglobin
Hct	Hematocrit
HD	Hemodialysis
HILIC-UPLC	Hydrophilic Interaction Ultra Performance Liquid Chromatography
HIV	Human Immunodeficiency Virus
HMWS	High Molecular Weight Species
HSA	Human Serum Albumin
IBD	International Birth Date
IND	Investigational New Drug (Application)
ITT	Intent-To-Treat (Population)
IU	International Unit
IV	Intravenous(ly)
K_D	Binding Affinity Constant
kg	Kilogram
k_{off}	Off-Rate
k_{on}	On-Rate
L	Liter
Lac	N-Acetyllactosamine
LC-MS	Liquid Chromatography-Mass Spectrometry
LMWS	Low Molecular Weight Impurities
LOQ	Limit Of Quantitation
LS	Least Square (Mean)
LTSS	Long-Term Safety Study
MAR	Missing At Random
Mcg or µg	Microgram
MedDRA	Medical Dictionary For Regulatory Activities

Abbreviation	Description
Met	Methionine
mFAS	Modified Full Analysis Set
mg	Milligram
mL	Milliliter
NAb	Neutralizing Antibody
Near-UV CD	Near Ultraviolet Circular Dichroism
NeuAc	N-Acetylneuraminic Acid
NeuGc	N-Glycolylneuraminic Acid
ng	Nanogram
NKF-KDOQI	National Kidney Foundation-Kidney Disease Outcome Quality Initiative
PD	Pharmacodynamic(s)
Phe	Phenylalanine
PHS	Public Health Service
PK	Pharmacokinetic(s)
PP	Per Protocol (Population)
PRCA	Pure Red Cell Aplasia
PT	Preferred Term
RBC	Red Blood Cell
REMS	Risk Evaluation And Mitigation Strategy
RET	Retained Set
Ret%	Reticulocyte Count As A Percentage Of Total Erythrocytes
rhEPO	Recombinant Human Erythropoietin
RIP	Radioimmunoprecipitation (Assay)
RP-HPLC	Reversed Phase High Performance Liquid Chromatography
RP-UPLC	Reversed Phase Ultra Performance Liquid Chromatography
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous(ly)
SD	Standard Deviation
Ser	Serine
SMQ	Standard MedDRA Queries
SPR	Surface Plasmon Resonance
$t_{½}$	Elimination Half-Life
T_m	Melting Temperature
TEAE	Treatment-Emergent Adverse Event
TIW	Three Times Per Week
TK	Toxicokinetic

Abbreviation	Description
Trp	Trytophan
Tyr	Tyrosine
TSAT	Transferrin Saturation

1. EXECUTIVE SUMMARY

1.1. Introduction

Hospira, a Pfizer company, has developed Epoetin Hospira (conditionally approved proprietary name, Retacrit™) as a proposed biosimilar product to the US-licensed reference product Epogen®/Procrit® (epoetin alfa) for treatment of the same indications currently approved for the reference product, namely:

- For the treatment of anemia due to chronic kidney disease (CKD), including patients on dialysis and not on dialysis, to decrease the need for red blood cell (RBC) transfusion;
- For the treatment of anemia due to zidovudine administered at ≤ 4200 mg/week in human immunodeficiency virus (HIV)-infected patients with endogenous serum erythropoietin levels of ≤ 500 mUnits/milliliter (mL);
- For the treatment of anemia in patients with non-myeloid malignancies where anemia is due to the effect of concomitant myelosuppressive chemotherapy, and upon initiation, there is a minimum of 2 additional months of planned chemotherapy;
- To reduce the need for allogeneic RBC transfusions among patients with perioperative hemoglobin (Hb) > 10 to ≤ 13 g/dL who are at high risk for perioperative blood loss from elective noncardiac, nonvascular surgery.

The Epogen/Procrit reference product was approved in the US in 1989. It is licensed to and marketed by Amgen Inc. (Epogen) and Janssen Products, LP (Procrit). As the first biosimilar to Epogen/Procrit submitted to and being reviewed by the Food and Drug Administration (FDA), and per FDA's request, this application is brought to the Oncologic Drugs Advisory Committee for consideration.

This briefing document presents a summary of the data demonstrating that Epoetin Hospira is biosimilar to the US-reference product Epogen/Procrit using the stepwise approach outlined in the FDA guidance. Specifically, this briefing document includes evidence to establish that Epoetin Hospira has highly similar physicochemical structure and biological function, equivalent pharmacokinetics (PK) and pharmacodynamics (PD), and comparable safety and efficacy to Epogen/Procrit to meet the statutory definition of "highly similar" with "no clinically meaningful differences". The totality of evidence in the Epoetin Hospira development program demonstrates the biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product and includes scientific justification for extrapolation to all current Epogen/Procrit indications in the US.

Regulatory Pathway

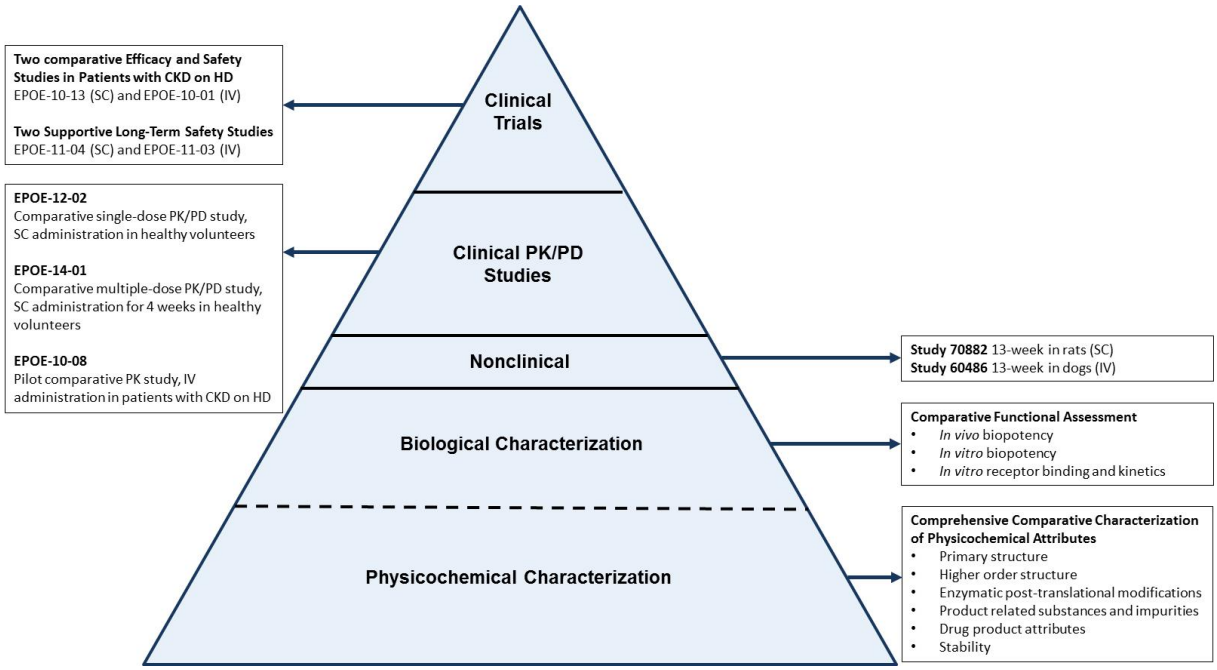
The Biologics Price Competition and Innovation (BPCI) Act of 2009 created an abbreviated licensure pathway for biological products shown to be "biosimilar" to an FDA-licensed biological product (the "reference product"). Section 351(k) of the amended Public Health Service (PHS) Act outlines the abbreviated pathway wherein a proposed biological product that is demonstrated to be biosimilar to a reference product can rely on certain existing

scientific knowledge about the safety, purity, and potency of the reference product to support licensure (described in Section 2.1). A stepwise approach in evaluating the evidence of biosimilarity is recommended during the development of a biosimilar product, beginning with the structural and functional characterization of both the proposed biosimilar product and the reference product. The robustness of the physicochemical and functional data aids in determining the extent and nature of both the nonclinical and clinical studies required to demonstrate biosimilarity. Ultimately, the evaluation of biosimilarity is based on the "totality of evidence" obtained from both analytical and clinical studies. Biosimilarity is demonstrated when "the product is highly similar to the reference product notwithstanding minor differences in clinically inactive components" and there are "no clinically meaningful differences between the proposed product and the reference product in terms of safety, purity and potency."

Overview of Epoetin Hospira Development Program

The Epoetin Hospira biosimilar development program includes comprehensive comparative analytical, nonclinical, and clinical studies (Figure 1), as recommended by the FDA to establish the biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product.

Figure 1. Overview of Sources of Epoetin Hospira Biosimilarity Data



The foundation of the Epoetin Hospira assessment of biosimilarity is the extensive physicochemical and functional characterization performed on both Epoetin Hospira and the Epogen/Procrit reference product. In total, 35 commercial-scale Epoetin Hospira Drug Product (DP) lots, 9 commercial-scale Epoetin Hospira Drug Substance (DS) lots and 54 reference product lots were evaluated. These lots were subjected to robust, orthogonal characterization testing to evaluate structure and functional activity. In particular,

determination of functionality and potency of Epoetin Hospira and the Epogen/Procrit reference product, using *in vitro* cell-based and receptor binding assays as well as an *in vivo* functional assay, was an important part of the analytical and pharmacologic demonstration of biosimilarity.

The nonclinical development program included two 13-week comparative Good Laboratory Practices (GLP)-compliant toxicity studies, one in rats and one in dogs. Both studies compared Epoetin Hospira with the reference product.

The clinical development program for Epoetin Hospira comprised three comparative pharmacokinetic (PK)/pharmacodynamic (PD) studies and four clinical comparative efficacy and safety studies (Table 1).

Table 1. Clinical Studies in the Epoetin Hospira Clinical Development Program

Study	Description	Route of Administration	Type/Number of Subjects
PK/PD Studies			
EPOE-12-02	Comparative single-dose PK/PD study	Subcutaneous	HS, 81 randomized
EPOE-14-01	Comparative multiple-dose PD/PK study	Subcutaneous	HS, 129 randomized
EPOE-10-08*	Pilot comparative PK study	Intravenous	CKD on HD; 105 randomized
Clinical Efficacy and Safety Studies			
EPOE-10-13	Comparative safety and efficacy study	Subcutaneous	CKD on HD; 320 randomized
EPOE-10-01	Comparative safety and efficacy study	Intravenous	CKD on HD; 612 randomized
EPOE-11-04	Supportive long-term safety study	Subcutaneous	CKD on HD; 173 enrolled
EPOE-11-03	Supportive long-term safety study	Intravenous	CKD on HD; 414 enrolled

CKD, chronic kidney disease; HD, hemodialysis; HS, healthy subjects.
* All studies, except EPOE-10-08, used the same late stage development formulation. EPOE-10-08 was a pilot PK study using an early formulation.

1.2. Biosimilarity Based on Results of Analytical Studies

Biosimilarity between Epoetin Hospira and the Epogen/Procrit reference product was demonstrated through a comprehensive analytical assessment consistent with the *FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (FDA 2015a). The analytical similarity assessment included extensive structural and functional characterization studies. Selection of the attributes evaluated in the

biosimilarity assessment was informed by a Critical Quality Attribute (CQA) assessment in which attributes were assigned a criticality level of high, medium, or low based on their impact to any of five dimensions: biological activity, PK/PD, clinical efficacy, immunogenicity, and safety/toxicity. High- and medium-criticality attributes are those with an established or potential link to patient safety and/or clinical performance, respectively. Low-criticality attributes do not impact patient safety or clinical performance and are designated as non-CQAs.

The comparative assessment included analysis of the data from the analytical similarity assessment using approaches of varying statistical rigor. The statistical approaches were applied based on a ranking system, consistent with FDA feedback, in which attributes were assigned to tiers commensurate with their potential clinical relevance and links to the mechanism of action. The focus on mechanism of action (Chow et al., 2016) for definition of the statistical tiers differs from the CQA assessment, where attributes are assigned a criticality level based on their impact to any of the five dimensions noted earlier.

Three statistical analysis tiers were defined consistent with FDA guidance, with the highest degree of statistical rigor applied to Tier 1. The two Tier 1 attributes in the Epoetin Hospira program were selected, in consultation with FDA, based on their relevance to the epoetin mechanism of action and potential clinical significance. Tier 2 attributes are high-criticality attributes for which the direct link to the mechanism of action is less certain or that are redundant relative to Tier 1 attributes. Tier 3 attributes are low-criticality attributes, those for which the data are not amenable to formal statistical comparison, or attributes that are redundant relative to Tier 2 attributes. An overview of the FDA statistical tier construct is provided in Table 2.

Table 2. Overview of FDA Tiered Statistical Analysis

Attribute Tier	Description	Statistical Treatment
1	**Most** relevant to • mechanism of action • function of product • clinical effects	Equivalence testing
2	**Potentially** relevant to • mechanism of action • function of product • clinical effects OR redundant to Tier 1 attributes	Evaluation versus reference product quality ranges (e.g., $Mean_{Ref} \pm 3SD$)
3	**Least** relevant to • mechanism of action • function of product • clinical effects OR redundant to Tier 2 attributes OR not amenable to quantitative comparisons	Raw data and graphical comparison

Attributes evaluated experimentally in the biosimilarity assessment include: primary structure, higher-order structure, post-translational modifications, product-related substances and impurities, drug product attributes, functional activity, and stability. This comparative testing was conducted using 33 state-of-the-art analytical methods to evaluate 35 commercial scale lots of Epoetin Hospira DP, 9 lots of Epoetin Hospira DS, and 54 lots of the Epogen/Procrit reference product. A summary of the key results from the analytical similarity assessment is presented in Table 3.

Table 3. Summary of Key Analytical Similarity Assessment Results

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Results/Discussion	Analytical Similarity Criteria Met
Primary Structure	Amino Acid Sequence	Qualitative comparison	Identical amino acid sequence	✓
	Sites of Glycosylation	Qualitative comparison	Same sites of glycosylation	✓
	Disulfide Mapping	Qualitative comparison	Same disulfide linkages	✓
Higher Order Structure	Melting temperature (T_m)	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range	✓
	Secondary structure	Tier 2 – Quality Range and Tier 3 – Qualitative comparison	Epoetin Hospira results within Epogen/Procrit quality range (Tier 2) Visually similar spectra[b] (Tier 3)	✓
	Tertiary structure	Tier 3 – Qualitative comparison	Visually similar[b] and similar λ_{max}	✓
Enzymatic Post-Translational Modifications	Total Sialic Acids	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range	✓
	N-glycolylneuraminic acid (NeuGc)[c]	Tier 3 – Qualitative comparison	Lower levels of non-human NeuGc sialic acid species in Epoetin Hospira	✓
	N-Linked Glycans: Sialic Acid Distribution	Tier 3 – Qualitative comparison	Same sialylated glycan structures as Epogen/Procrit	Same structures observed with minor quantitative differences[d]
	N-Linked Glycans: Di-, Tri- and Tetra-Antennary Structures	Tier 3 – Qualitative comparison	Same antennary glycan structures as Epogen/Procrit	

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Results/Discussion	Analytical Similarity Criteria Met
Product Related Substances and Impurities	Dimer and Other High Molecular Weight Species (HMWS)	Tier 3 – Qualitative comparison	Epoetin Hospira levels of HMWS consistent with levels in Epogen/Procrit	✓
	Deamidation at Asn 147	Tier 2 – Quality range	Minor quantitative differences: Epoetin Hospira results range from 0.4 to 1.5%; Epogen/ Procrit quality range is 0.3 to 0.7%.	Results for both products < 1.5%; no impact on biopotency (see Section 4.3.4.2)
	T2=T20 Trisulfide at Cys7-Cys161	Qualitative comparison	Similar	✓
Drug Product Attributes	Epoetin Content	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range[e]	✓
	Container Volume	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range	✓
	Particulate Matter	Qualitative comparison	Levels of particulates substantially lower (more favorable) in Epoetin Hospira	✓
Functional Attributes	*In Vivo* Biopotency (Normocythaemic mouse)	Tier 1 – Equivalence Testing	Epoetin Hospira is equivalent to Epogen/Procrit	✓
	In Vitro Specific Activity	Tier 1 – Equivalence Testing	Epoetin Hospira is equivalent to Epogen/Procrit	✓
	In Vivo Specific Activity	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range	✓
	Competitive Receptor Binding	Tier 2 – Quality range	Epoetin Hospira results within Epogen/Procrit quality range	✓
	Receptor Binding Affinity and Kinetics	Tier 3 – Qualitative comparison	Epoetin Hospira results are consistent with Epogen	✓

[a] Quality ranges for Tier 2 attributes represent ±3SD of the reference product mean. The equivalence margin for Tier 1 attributes is ± 1.5SD of the reference product mean.

[b] Visually similar: Similar spectral features, including position and magnitude of spectral minima and/or maxima. No new peaks or bands greater than the limit of detection of the method.

[c] N-glycolylneuraminic acid (NeuGc) is a non-human sialic species that may be present at low levels in sialylated glycoproteins expressed in non-human cell lines.

[d] Minor differences in the relative abundance of glycan structures were evaluated extensively using *in vitro* bioassays and an *in vivo* mouse PD model (normocythaemic mouse). These *in vitro* and *in vivo* studies demonstrated conclusively that any quantitative differences observed in the epoetin glycan profile do not impact the binding affinity or binding kinetics of Epoetin Hospira to the epoetin receptor or the *in vivo* half-life and biological activity of Epoetin Hospira relative to the Epogen/Procrit reference product. These *in vitro* and *in vivo* studies are also supported by the comparative nonclinical and clinical study results described in Section 5 and Section 6 which utilized Epoetin Hospira and Epogen lots having these minor differences.

[e] Results for the Epoetin Hospira lots manufactured following the epoetin content target change described in Section 3.2.

Comparative analysis of the primary structure of the epoetin present in Epoetin Hospira and the Epogen/Procrit reference product demonstrate that the amino acid sequence of the epoetin protein in Epoetin Hospira is identical to that in the Epogen/Procrit reference product. The peptide mapping results confirm that the sites of N- and O-linked glycosylation are identical for Epoetin Hospira and the Epogen/Procrit reference product. The disulfide bonds are also the same between the two products.

Evaluation of secondary and tertiary structure is an essential component of the analytical assessment of biosimilarity to confirm that the epoetin present in Epoetin Hospira is folded in a manner similar to the epoetin present in the Epogen/Procrit reference product. Complementary spectral methods were used to compare the secondary and tertiary structure of the products. The results demonstrate that the secondary and tertiary structures of Epoetin Hospira are similar to the Epogen/Procrit reference product.

Equivalence was demonstrated between Epoetin Hospira and the reference product for the *In Vivo* Biopotency and *In Vitro* Specific Activity Tier 1 attributes. *In Vivo* Biopotency represents the most clinically relevant bioassay as it is linked to the mechanism of action and pharmacodynamics (PD) of the epoetin present in Epoetin Hospira and the Epogen/Procrit reference product. *In Vitro* Specific Activity represents the most precise measure for assessing the impact of any minor quantitative physicochemical differences observed between Epoetin Hospira and the reference product on the inherent activity of epoetin in these products.

Overall, the results of the analytical similarity assessment demonstrate that Epoetin Hospira is highly similar to the US-licensed Epogen/Procrit reference product.

1.3. Biosimilarity Based on Results of Nonclinical Assessments

The Epoetin Hospira nonclinical program included two GLP-compliant 13-week repeat-dose comparative toxicity studies (including PD, PK/toxicokinetic [TK], and immunogenicity evaluations) – one in rats and one in dogs – to support the demonstration of biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product.

The overall findings from the two comparative GLP-compliant toxicity studies with the incorporation of PD, PK/TK, and immunogenicity assessments demonstrate that Epoetin Hospira and Epogen produced similar effects in rats and dogs. Of note, a species difference was observed in the rat with regard to PK/TK and PD under subcutaneous conditions where human serum albumin (HSA) is an excipient uniquely in the reference product. The use of HSA in the reference product likely contributed to increased immunogenicity for Epogen reference product in the rat, thereby influencing the comparative PK, TK and PD in the rat. This was not seen in dogs under intravenous (IV) conditions where the PK/TK and PD were similar between the two treatment arms. The clinical data better inform the assessment of PK/PD similarity. Results from these studies support the safety of Epoetin Hospira for the intended clinical use. The comparative nonclinical data provides additional support for the demonstration of biosimilarity between Epoetin Hospira and the Epogen reference product.

1.4. Clinical Pharmacology Similarity

PK/PD similarity between Epoetin Hospira and Epogen reference product was demonstrated under single and multiple fixed-dose conditions in healthy subjects in studies EPOE-12-02 and EPOE-14-01, respectively.

Single-dose and multiple-dose PK/PD studies conducted in healthy subjects are the most discerning studies for characterizing the PK and PD responses to epoetin and for identifying any potential differences between products, should they exist. Healthy subjects lack comorbidities and concomitant medications that may confound PK results. Healthy subjects also maintain functional bone marrow that might otherwise confound PD results.

The PK and PD are well established for Epogen in multiple patient populations and healthy subjects. Demonstration of PK and PD equivalence of Epoetin Hospira and Epogen in healthy subjects provides the evidence that equivalent PK and PD profiles for the two products can be expected in all populations and conditions of use.

1.4.1. Single Dose PK/PD: Study EPOE-12-02

Study EPOE-12-02 was designed as a single-center, open-label, randomized, 2-period, 2-sequence crossover. Eighty-one healthy male subjects were randomized to receive a single 100 U/kg dose of either Epoetin Hospira or Epogen, administered subcutaneously (SC), on Day 1 of Period 1 or Day 1 of Period 2, according to the subject's randomized sequence. Pharmacokinetics was the primary endpoint based on the following parameters:

- Maximum observed concentration (C_{max}) determined from baseline-adjusted epoetin concentrations (BAEC) and
- Area under the concentration-time curve from time zero to time of last measurable concentration (AUC_{0-t}).

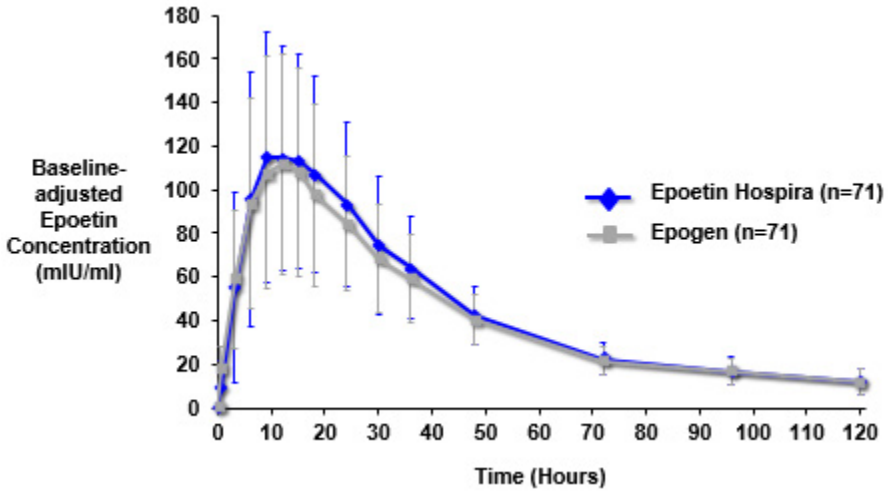
Pharmacodynamics was a secondary endpoint based on the following parameters:

- Area under the effect-time curve from time zero to time of last measurable reticulocyte count ($AUEC_{0-t}$) and maximum observed effect (E_{max}) determined from reticulocyte count as percent of erythrocytes (Ret%).

Single-Dose Pharmacokinetics

Mean BAEC profiles over time after single SC administration of 100 U/kg of Epoetin Hospira or Epogen were similar between the treatment groups (Figure 2).

Figure 2. Single Dose PK: Baseline-Adjusted Epoetin Concentration Profiles After Single SC Administration of 100 U/kg of Epoetin Hospira or Epogen to Healthy Male Subjects in the Single Dose PK/PD Study (Pharmacokinetic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing was at Time 0.
Study EPOE-12-02.

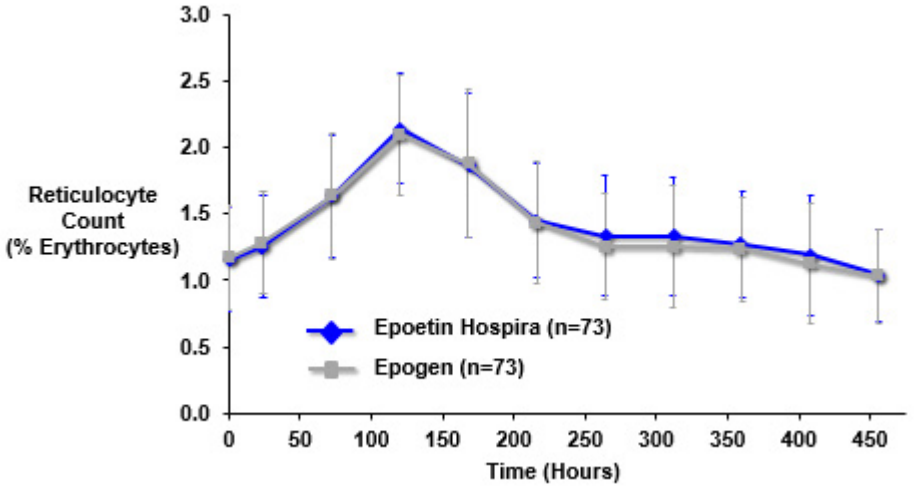
Pharmacokinetic similarity between Epoetin Hospira and Epogen following single-dose administration was demonstrated based on 90% confidence intervals (CIs) for the geometric mean ratios (GMRs) of Epoetin Hospira to Epogen (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both C_{max} derived from BAEC in serum (ratio = 1.09, 90% CI: 1.01, 1.18) and AUC_{0-t} (ratio = 1.05, 90% CI: 1.01, 1.11). Pharmacokinetic similarity was further assessed by multiple sensitivity analyses which support the primary analysis conclusions.

Single-Dose Pharmacodynamics

Time profiles of Ret% were similar between the Epoetin Hospira and Epogen treatments over a 20-day period following single-dose study drug administration (Figure 3). Reticulocyte count (expressed as percent of erythrocytes) is a well-established PD marker reflective of the mechanism of action of epoetin on erythropoietic response and a measure of therapeutic effect, and is therefore an appropriate PD parameter.

Pharmacodynamic results of Study EPOE-12-02 are consistent with the findings of previously published work, reporting an increase in reticulocyte count within 3 to 4 days with a return to baseline by approximately 22 days following SC administration of recombinant human erythropoietin to healthy subjects (Cheung et al., 1998; Ramakrishnan et al., 2004).

Figure 3. Single Dose PD: Ret% after Single Subcutaneous Administration of 100 U/kg of Epoetin Hospira or Epogen in the Single Dose PK/PD Study (Pharmacodynamic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing at Time 0.
Study EPOE-12-02.

Pharmacodynamic similarity between Epoetin Hospira and Epogen following single-dose administration was demonstrated based on FDA-requested 90% CIs for geometric mean ratio (GMRs) (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both $AUEC_{0-t}$ derived from Ret% (ratio = 1.01; 90% CI: 0.98, 1.05) and E_{max} derived from Ret% (ratio = 1.02; 90% CI: 0.99, 1.05), and was supported by findings from sensitivity analysis conducted in subjects who received at least one dose of study drug (data included in the Biologics License Application [BLA]). Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated based on pre-specified 95% CIs for GMRs ($AUEC_{0-t}$: 95% CI: 0.98, 1.05 and E_{max}: 95% CI: 0.98, 1.06).

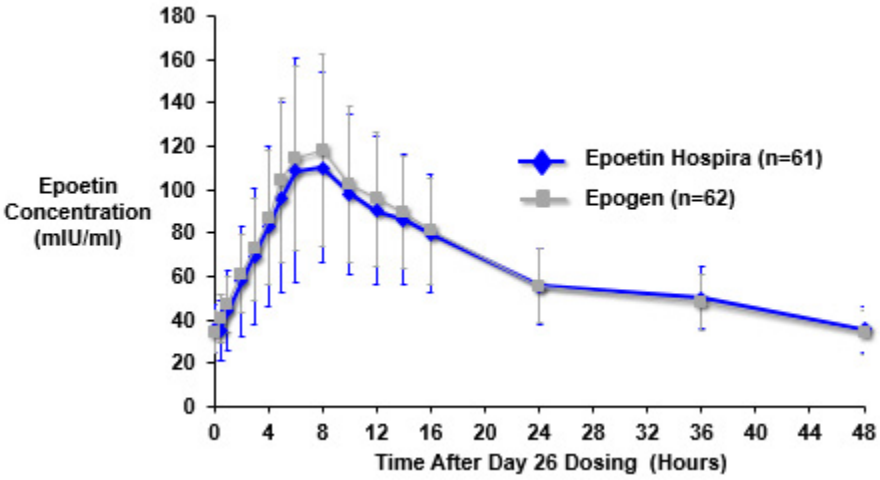
1.4.2. Multiple Dose PK/PD Results: Study EPOE-14-01

Study EPOE-14-01 was designed as a single-center, open-label, randomized, parallel group study that enrolled 129 healthy male subjects. One-hundred-twenty-nine subjects were randomized to receive either Epoetin Hospira or Epogen 100 U/kg SC three times weekly (TIW) for 4 weeks. The PD primary endpoint was area under the effect curve for hemoglobin ($AUEC_{Hb}$). Pharmacokinetic parameters of area under the concentration-time curve from time zero to 48 hours (AUC_{0-48}) and C_{max} were secondary endpoints.

Multiple-Dose Pharmacokinetics

Mean (± SD) epoetin concentration-time profiles were similar between the Epoetin Hospira and Epogen treatment groups on Day 26 (Figure 4).

Figure 4. Multiple Dose PK: Serum Epoetin Concentration Profiles over Time on Day 26 after Subcutaneous Administration of 100 U/kg TIW for 4 Weeks of Epoetin Hospira or Epogen to Healthy Male Subjects in the Multiple Dose PK/PD Study (Pharmacokinetic Population)



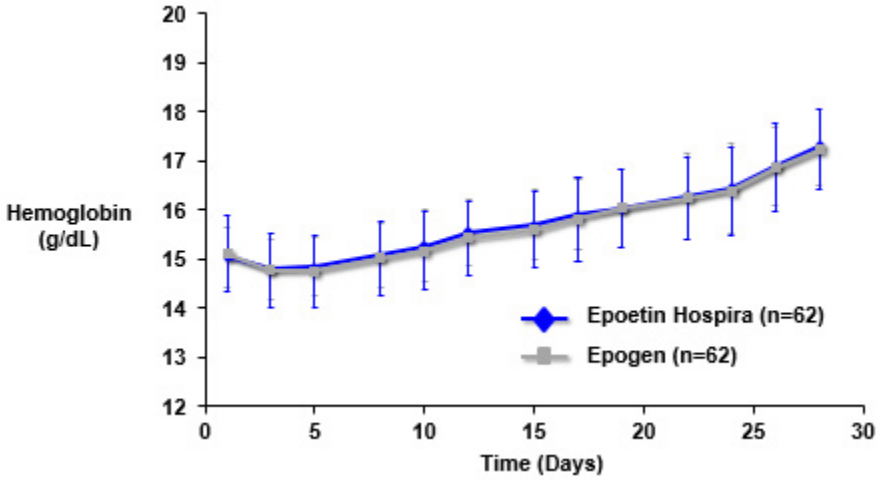
Values are shown as mean with bars representing ± 1 SD. Dosing was at Time 0 on Day 26.
Study EPOE-14-01.

Pharmacokinetic similarity between Epoetin Hospira and Epogen following multiple-dose administration was demonstrated based on 90% CIs for the GMRs (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both AUC_{0-48} (ratio = 0.974; 90% CI: 0.896, 1.059) and C_{max} (ratio = 0.938; 90% CI: 0.839, 1.049). Pharmacokinetic similarity was further assessed by multiple sensitivity analyses which support the primary analysis conclusions.

Multiple-Dose Pharmacodynamics

Hemoglobin-time profiles from baseline through Day 28 were similar between the Epoetin Hospira and Epogen treatment groups (Figure 5). Like reticulocyte count, hemoglobin (Hb) is a well-established PD marker, reflective of the known mechanism of action of epoetin on erythropoietic response and a measure of therapeutic effect, and is therefore an appropriate PD parameter. The Hb response may take 4 weeks or longer of multiple dosing to manifest compared to reticulocyte count (Cheung et al., 2001; Ramakrishnan et al., 2004; Sorgel et al., 2009), therefore Hb response and consistency over time is best evaluated in a multiple-dose study.

Figure 5. Multiple Dose PD: Hemoglobin over Time Profile after Multiple-Dose Subcutaneous Administration of Epoetin Hospira or Epogen in the Multiple Dose PK/PD Study (Pharmacodynamic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing at Time 0.
Study EPOE-14-01.

Pharmacodynamic similarity between Epoetin Hospira and Epogen following multiple-dose administration was demonstrated based on the FDA-requested 90% CI for GMR (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.965 – 1.035 for $AUEC_{Hb}$ (ratio = 1.006; 90% CI: 0.998, 1.015), and was supported by sensitivity analysis conducted in subjects who received at least one dose of study drug (data included in the BLA). The PD equivalence margin was assessed considering the range of Hb values specified at entry of 13.0 to15.5 g/dL (midpoint 14.2 g/dL) and the equivalence margin established in the literature for Hb and also employed in the comparative efficacy studies for Epoetin Hospira. The corresponding equivalence acceptance range calculated as a percent is ± (0.5/14.2) x 100 = ± 3.5%. In this analysis, an analysis of covariance (ANCOVA) model was used, with baseline Hb as a covariate and treatment group as a factor. Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated for $AUEC_{Hb}$ based on the pre-specified 95% CIs for GMRs (0.996, 1.016).

1.5. Clinical Efficacy Similarity

1.5.1. Study Design

Two comparative clinical efficacy and safety studies established similar efficacy between Epoetin Hospira and Epogen in support of a demonstration of biosimilarity. The equivalence studies are generally described in Table 1 (Section 1.1) with important design characteristics summarized below:

- Comparative efficacy and safety during subcutaneous administration: (Study EPOE-10-13): multicenter, double-blind, randomized, parallel-arm, active control (Epogen) 16-week study.

- Comparative efficacy and safety during intravenous administration (Study EPOE-10-01): multicenter, double-blind, randomized, parallel-arm, active control (Epogen) 24-week study

Both studies enrolled subjects with chronic kidney disease (CKD), thereby using the most sensitive population to evaluate clinically meaningful differences between Epoetin Hospira and Epogen, should they exist. Epoetin deficiency is the predominant factor in anemia secondary to chronic kidney failure. The CKD on hemodialysis (HD) population is the most epoetin-deficient across the approved conditions of use for Epogen, and therefore, the most likely to reveal potential efficacy differences between products. In addition, this population is sufficiently immunocompetent across the various conditions of Epogen clinical use, thereby the most sensitive for the assessment of immunogenicity.

Comparative Efficacy and Safety during Subcutaneous Administration (Study EPOE-10-13)

Key enrollment criteria were as follows:

- 18 to 80 years old,
- on stable intravenous (IV) or subcutaneous (SC) Epogen treatment (≤ 600 U/kg/week),
- stable Hb (mean between 9.0 and 11.0 g/dL) for 4 weeks prior to randomization into Maintenance Period,
- on stable, adequate hemodialysis (HD) for at least 12 weeks prior to randomization into Maintenance Period,
- adequate iron stores, and
- no history of disorders that affect red blood cells (RBC).

Eligible subjects (described in Section 6.2.2.1) were randomized (1:1) to Epoetin Hospira or Epogen in a Dose Stabilization Period and required to have a stable SC dosing before a second randomization (1:1) to Epoetin Hospira or Epogen into the Maintenance Period (see Figure 36 for study design diagram).

Subjects who had been on SC Epogen at the time of Screening and had demonstrated protocol-defined optimal stable dosing were randomized into the Dose Stabilization Period, and then were immediately re-randomized into the Maintenance Period; they received no treatment with study drug during the Dose Stabilization Period.

Subjects who had been on SC Epogen at the time of Screening but did not meet the protocol-defined optimal stable dosing criteria were randomized into the 12- to 18-week Dose Stabilization Period to achieve at least 4 weeks of optimal stable SC dosing, which was required to qualify for entry into the Maintenance Period.

Subjects who had been on IV Epogen prior to study enrollment were transitioned to SC Epogen during the Dose Stabilization Period. For these subjects, the SC dose was reduced an initial 20 to 30% from the IV weekly dose they received during the last week of the

up-to-4-week Screening Period. Subjects were then randomized into the 12- to 18-week Dose Stabilization Period to achieve at least 4 weeks of optimal stable dosing, which was required to qualify for entry into the Maintenance Period.

During the study, the dose of study drug was evaluated for adjustment on a regular basis (i.e., at least every week) to maintain the Hb value within a range of 9.0 to 11.0 g/dL. Adjustments to dose for study treatment were allowed in line with the approved Epogen US Package Insert (Epogen PI, 2014).

After completing the Maintenance Period, eligible subjects could enter the open-label long-term safety study (LTSS), EPOE-11-04 (SC administration), to be treated with Epoetin Hospira for up to an additional 48 weeks.

Comparative Efficacy and Safety during Intravenous Administration (Study EPOE-10-01)

Eligible subjects (identical to those enrolled in Study EPOE-10-13 [SC]) were randomized in a 1:1 ratio to either Epoetin Hospira or Epogen; IV bolus injections were administered 1 to 3 times per week at the same stable weekly dose that the subject received during the last week of the up-to-4-week Screening Period. Subjects were treated for up to 24 weeks in the Treatment Period. During the study, investigators adjusted the dose, as needed, to maintain subjects' Hb within a range of 9.0 to 11.0 g/dL, using the same guidelines as those followed in Study EPOE-10-13 (SC).

After completing the Treatment Period, eligible subjects could enter LTSS EPOE-11-03 (IV administration) and be treated with Epoetin Hospira for up to an additional 48 weeks.

1.5.2. Comparative Efficacy Results

The majority of subjects who participated in Study EPOE-10-13 (SC) (86%) or Study EPOE-10-01 (IV) (84%) completed the study. The demographics and baseline disease characteristics of randomized subjects are representative of the population of CKD patients on HD (USDS, 2013), and the Epoetin Hospira and Epogen groups were well matched in each study.

Both comparative studies, EPOE-10-13 (SC) and EPOE-10-01 (IV), met their co-primary endpoints for efficacy by demonstrating equivalence between Epoetin Hospira and the Epogen reference product, when administered SC or IV, in mean weekly Hb level maintained and mean weekly dose administered to maintain Hb within the target range of 9.0 to 11.0 g/dL. The Sponsor pre-specified 95% CIs for the difference between Epoetin Hospira and Epogen in mean weekly Hb and mean weekly dose during the last 4 weeks of the nominal treatment period (defined as the 16-week Maintenance Period in Study EPOE-10-13 [SC] and the 24-week Treatment Period in Study EPOE-10-01 [IV]) were within the pre-specified equivalence limits of ± 0.5 g/dL and ± 45 U/kg/week, respectively (Table 4). The FDA during the 2017 BLA review subsequently requested 90% CIs be used. Both the 90% CIs and the 95% CIs are provided in the displays for clarity.

Because the treatment goal is to maintain Hb levels within the desired therapeutic range using the epoetin dose, comparison of Epoetin Hospira and Epogen for both dose and the resulting Hb levels are the most appropriate efficacy measures to perform comparative efficacy assessments between the two products. The use of these two endpoints is a well-characterized standard method of assessing comparative efficacy of proposed biosimilar erythropoiesis-stimulating agents (ESAs) and reference products (Wizemann et al., 2008; Krivoshiev et al., 2008; Krivoshiev et al., 2010).

In secondary endpoint assessments, mean weekly Hb and mean epoetin dose were similar between the Epoetin Hospira and Epogen treatment groups for each week during the nominal treatment period in both studies (refer to Figure 40 and Figure 41, in Section 6.2.5.4). The extent of blood transfusions at any time point during the study was also similar between Epoetin Hospira and Epogen treatment groups (4% in each treatment group in Study EPOE-10-13 [SC]; 6% in each treatment group in Study EPOE-10-01 [IV]) adding consistency to the findings with the primary and secondary endpoints. Overall, blood transfusions occurred in a minority of subjects.

Table 4. Mean Weekly Hemoglobin and Mean Weekly Dose per Kilogram Body Weight during the Last 4 Weeks of the Nominal Treatment Period (Intent-to-Treat Population)

Parameter	Statistic	Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)			Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)		
		Epoetin Hospira (N=124)	Epogen (N=122)	Difference	Epoetin Hospira (N=306)	Epogen (N=306)	Difference
Mean Weekly Hb (g/dL)	LS Mean (SE)	10.16 (0.073)	10.12 (0.074)	0.04 (0.104)	10.17 (0.047)	10.28 (0.047)	-0.12 (0.066)
	90% CI*			(-0.13, 0.21)[a]			(-0.22, -0.01)[a]
	95% CI**			(-0.17, 0.24)			(-0.25, 0.01)
Mean Weekly Dose (U/kg)	LS Mean (SE)	79.57 (4.356)	81.91 (4.373)	-2.34 (6.175)	90.16 (3.874)	89.79 (3.880)	0.37 (5.483)
	90%CI*			(-12.54, 7.85)[b]			(-8.67, 9.40)[b]
	95% CI**			(-14.51, 9.82)			(-10.40, 11.13)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program

a. Equivalence is concluded if the 90% confidence interval of the LS Mean of the difference is contained within -0.5 and 0.5 g/dL.
b. Equivalence is concluded if the 90% confidence interval of the LS Mean of the difference is contained within -45 and 45 U/kg/week.

Note: LS Means and confidence intervals come from an ANCOVA model with fixed effect of treatment and baseline as a covariate.
Note: Using a hierarchical test strategy, equivalence of mean weekly Hb level was tested first. If equivalence was concluded, then equivalence of mean weekly dose per kg body weight was tested. If equivalence was concluded for both endpoints, then equivalence in efficacy between Epoetin Hospira and Epogen was concluded.
Note: Nominal treatment period is the 16-week Maintenance Period in Study EPOE-10-13 and/or the 24-week Treatment Period in Study EPOE-10-01.

1.6. Clinical Safety Assessment

Safety data were pooled to create a combined EPOE-10-13 (SC) and EPOE-10-01 (IV) randomized Epogen treatment group and a combined EPOE-10-13 (SC) and EPOE-10-01 (IV) randomized Epoetin Hospira treatment group. The integrated analysis of safety in the combined randomized clinical studies of over 800 subjects indicates that the safety profiles with Epoetin Hospira and the Epogen reference product administered SC or IV in the comparative studies in subjects with CKD are consistent, supporting demonstration of biosimilarity. Subjects who completed the 16-week Maintenance Period in EPOE-10-13 were eligible to enroll in the 48-week LTSS EPOE-11-04, and subjects who completed the 24-week Treatment Period in EPOE-10-01 were eligible to enroll in the 48-week LTSS EPOE-11-03. The cumulative safety data demonstrated that Epoetin Hospira was safe and well-tolerated when administered for up to 64 weeks or up to 72 weeks by SC and IV administration, respectively.

The incidences of adverse events (AEs) were comparable between the combined randomized treatment groups across all categories (Table 5).

Table 5. Overview of Adverse Events for Combined Randomized Treatment Groups

Subjects with:	Epoetin Hospira Randomized (N = 423) n (%)	Epogen Randomized (N = 426) n (%)
≥ 1 TEAE	321 (75.9%)	318 (74.6%)
≥ 1 SAE	101 (23.9%)	116 (27.2%)
Discontinued study drug due to a TEAE	13 (3.1%)	15 (3.5%)
Fatal event	9 (2.1%)	9 (2.1%)

TEAE, treatment-emergent adverse event; SAE, serious adverse event

The most frequently reported treatment-emergent adverse events (TEAEs) for Epoetin Hospira were nausea (9.5% vs. 7.7% of subjects in the randomized Epogen group), arteriovenous fistula site complication (7.6% and 7.0%, respectively), and vomiting (7.6% and 4.9%, respectively).

Investigators considered all deaths in the combined randomized treatment groups as either not or probably not related to study drug. Serious adverse events (SAEs) were reported in a comparable proportion of subjects in the randomized Epoetin Hospira and randomized Epogen groups (Table 5). The most frequently reported SAEs for Epoetin Hospira were pneumonia (1.7% vs. 2.3% of subjects in the randomized Epogen group), congestive cardiac failure (1.2% and 1.2%, respectively), and osteomyelitis (1.2% and 0.2%, respectively).

Events of interest (i.e., events based on the mechanism of ESA, which are summarized in the Warnings and Precautions section of the Epogen US Package Insert [Epogen PI, 2014]) were evaluated as part of the comparative safety assessment. The combined randomized Epoetin Hospira treatment group and the combined randomized Epogen treatment group showed comparable incidence of events of interest, including thromboembolic events (7.8% and 6.1%, respectively [Table 40]), hypertension (6.6% and 4.9%, respectively [Table 41]), potential allergic reactions (2.4% and 1.4%, respectively [Table 42]), myocardial infarction (0.9% and 0.7%, respectively), cerebrovascular events (0.9% and 1.4%, respectively), seizures (0.2% and 0.2%, respectively), and pure red cell aplasia (PRCA) (0% and 0%, respectively).

Laboratory data, vital sign data, and other safety assessments support comparable safety profiles between Epoetin Hospira and Epogen.

The potential for new safety signals after prolonged exposure was examined for both the SC and IV routes of administration in open-label LTSS. Based on data from the long-term safety studies, there were no new safety signals identified. The LTSS provide additional data that the safety profile of Epoetin Hospira is consistent with that historically seen with the reference product, Epogen.

1.7. Immunogenicity

A systematic, program-wide evaluation of clinical immunogenicity was performed in the Epoetin Hospira development program. Serum samples collected prior to the first dose of study drug and at pre-specified intervals throughout the study were evaluated using updated validated assays with stringent cut points to detect antibody formation against the reference product as well as Epoetin Hospira.

The incidence of anti-drug antibody (ADA)-positive subjects at any time during the treatment period was consistent between Epoetin Hospira (4 subjects [1.0%]) and Epogen (4 subjects [1.0%] (Table 43). Across the entire clinical program, neutralizing antibodies against recombinant human epoetin (rhEPO) were not detected in any subject.

The incidence rates of potential allergic reactions were similar between Epoetin Hospira and Epogen. None of the potential allergic reactions were medically determined to be hypersensitivity reactions suggestive of an immune response to epoetin. There were no reported events of PRCA in the clinical program. The immunogenicity profile of Epoetin Hospira is comparable to that of Epogen.

1.8. Extrapolation of Evidence for Biosimilar to All Epogen/Procrit Reference Product Indications

In line with *FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (FDA 2015a), the totality of evidence, as summarized in this Briefing Document, supports a demonstration of biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product, including comparative clinical data in CKD patients on HD. Additional indications for the reference product include treatment of anemia in adult patients with CKD not on dialysis; treatment of anemia in zidovudine-treated HIV-infected adult patients; and treatment of anemia in myelosuppressive chemotherapy-treated adult patients, as well as other conditions of use. Also in line with the FDA Guidance, a robust scientific justification is provided for extrapolation of information regarding the safety, purity, and potency of Epogen/Procrit in its additional licensed conditions of use to the proposed Epoetin Hospira biosimilar product. Specific considerations and how they are addressed are provided below.

- Mechanism of action in each condition of use:
  - Relative or absolute erythropoietin deficiency contributes to anemia in all approved indications for Epogen/Procrit.
  - The mechanism of action to stimulate erythropoiesis is common to all indications for Epogen/Procrit reference product (Jelkmann, 2007).
  - Comparative analytical biosimilarity functional assay results support same mechanism of action of Epoetin Hospira and Epogen/Procrit reference product.

- Pharmacokinetics and Pharmacodynamics:
  - There is a well characterized PK/PD relationship that generalizes across multiple epoetin products in healthy subjects and across all patient populations for which Epogen/Procrit reference product is indicated.
  - PK/PD equivalence was established between Epoetin Hospira and Epogen under single-dose and multiple-dose conditions.
- Expected toxicities, including immunogenicity:
  - Safety evaluation was conducted in CKD, which is the most sensitive model, as historical risk of PRCA is greatest in this population that also tends to be less immunocompromised than other conditions such as chemotherapy-induced anemia (CIA).
  - There is a well-characterized safety profile of Epogen/Procrit reference product across indications primarily driven by PD response that was equivalent between Epoetin Hospira and Epogen in comparative single-dose and multiple-dose PK/PD studies.
  - Similar comparative safety of Epoetin Hospira and Epogen reference product was observed in two sensitive populations: CKD on HD under SC and IV conditions and in healthy subjects under SC conditions.
- The establishment of PD similarity in healthy subjects under single and multiple dose conditions provides direct clinical evidence of equivalence in this non-anemic target population. The healthy subject population is representative of the population for whom the product is indicated for reduction of allogeneic RBC transfusions in patients undergoing elective, noncardiac, nonvascular surgery.

The totality of evidence along with the scientific justification data support extrapolation to all other indications currently approved for the Epogen/Procrit reference product.

1.9. Risk Evaluation and Mitigation Strategy

FDA recently communicated in April 2017 a change in requirements for Risk Evaluation and Mitigation Strategy (REMS) for erythropoiesis-stimulating agents (ESAs).
Specifically, FDA determined that the ESA REMS, which was limited to the use of Epogen/Procrit and Aranesp to treat patients with anemia due to associated myelosuppressive chemotherapy, is no longer necessary to ensure that the benefits of Epogen/Procrit and Aranesp outweigh its risks of shortened overall survival and/or increased risk of tumor progression or recurrence in patients with cancer. Pfizer is committed to working with FDA to ensure robust pharmacovigilance measures for Epoetin Hospira aligned with current FDA expectations and consistent with the Epogen/Procrit reference product and ESA class.

1.10. Summary of Evidence for Biosimilarity

Collectively, the data from the Epoetin Hospira development program demonstrate that Epoetin Hospira is highly similar to the Epogen/Procrit reference product and there are no

clinically meaningful differences between the proposed biosimilar and the reference product in terms of the safety, purity, and potency of the product. The statutory requirements to demonstrate biosimilarity have been met conclusively with the foundational bioanalytical studies and the definitive clinical PK/PD studies, which were supported by the clinical efficacy and safety studies including the program-wide immunogenicity assessment.

Pfizer followed FDA's stepwise approach in generating the data to demonstrate biosimilarity of Epoetin Hospira to Epogen/Procrit. Consistent with this approach, any residual uncertainty due to minor differences observed between Epoetin Hospira and Epogen/Procrit at any stage was evaluated and informed the following steps so as to address that uncertainty. A brief summary of the minor differences observed and how these potential residual uncertainties were addressed across the totality of evidence in the BLA is provided in Table 6. Ultimately, the analytical, nonclinical and clinical evaluation demonstrates that Epoetin Hospira is highly similar with no clinically meaningful differences to the Epogen/Procrit reference product.

Table 6. Minor Differences Observed between Epoetin Hospira and Epogen/Procrit and How Addressed

Category	Observed Difference	Potential Impact	How Addressed
Analytical - Physicochemical Structure	Relative abundance of some N- and O-glycan structures (Section 4.3.3)	PK/PD	▪ Functional testing using a mouse PD model demonstrates no PD impact ▪ Equivalence demonstrated in single-dose (EPOE-12-02) and multiple dose (EPOE-14-01) PK/PD studies
Analytical - Physicochemical Structure	Cys29-Cys33 trisulfide (Table 13)	Biopotency Immunogenicity	▪ Functional testing results demonstrate no impact on *in vitro* specific activity and *in vivo* biopotency ▪ No differences observed in immunogenicity in clinical studies
Analytical - Physicochemical Structure	Deamidated Asn147 (Section 4.3.4.2)	Biopotency Immunogenicity	▪ Functional testing results demonstrate no impact on *in vitro* specific activity and *in vivo* biopotency ▪ No differences observed in immunogenicity in clinical studies
Nonclinical - Comparative 13-week Rat SC Toxicity Study	PD and PK/TK (Section 5)	PK/PD Immunogenicity	▪ Equivalence demonstrated in single-dose (EPOE-12-02) and multiple dose (EPOE-14-01) PK/PD studies ▪ No differences observed in immunogenicity in clinical studies

Pfizer also addressed FDA information requests and a Complete Response (CR) letter issued by FDA as part of the review of the Epoetin Hospira BLA. FDA requested additional data and sensitivity analyses to align with the most current FDA expectations and to ensure the robustness of the data demonstrating biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product. A high-level summary of the additional data and analyses in the BLA is provided in Table 7. These sensitivity analyses are consistent with the pre-specified analyses and further support the conclusion that biosimilarity has been demonstrated between Epoetin Hospira and the Epogen/Procrit reference product.

Table 7. Additional Data and Sensitivity Analyses Requested by FDA

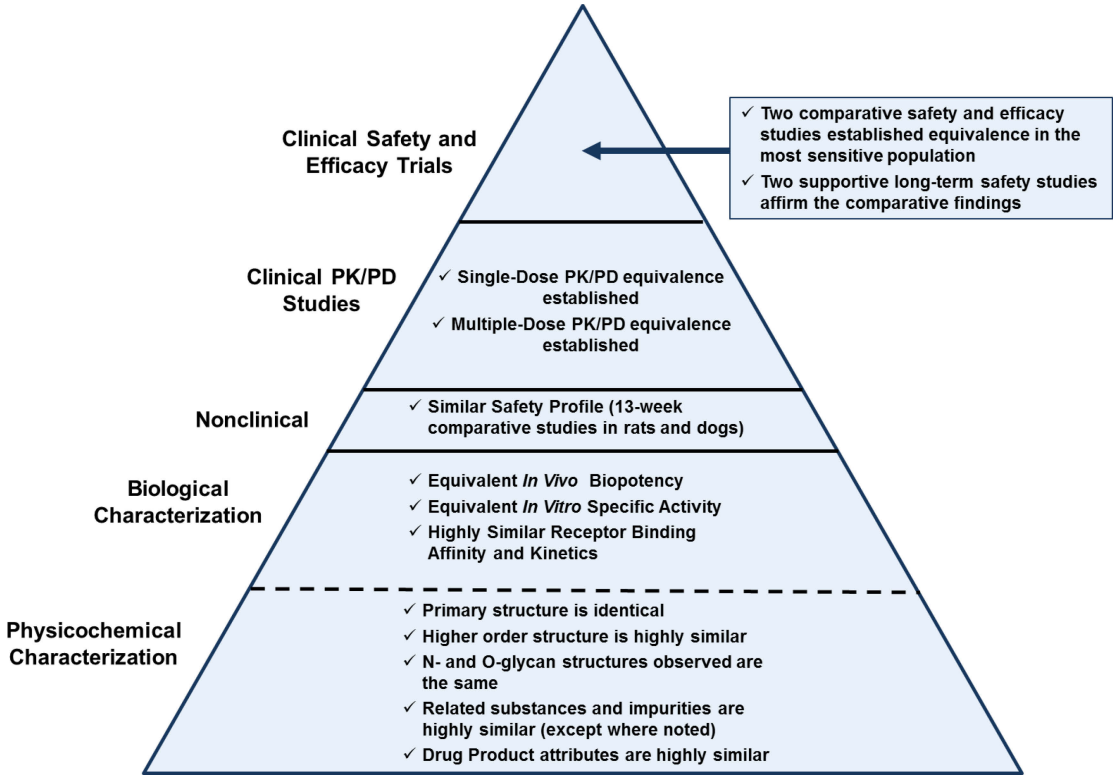
Category	Request	How Addressed
Manufacturing Process	Minor adjustment of the Epoetin Hospira Drug Product (DP) manufacturing process content target to more closely match epoetin protein content of the reference product	▪ Revised target implemented during the BLA review; nine (9) lots of Epoetin Hospira DP (three lots at each of the 2000, 10,000 and 40,000 U/mL dose strengths) were manufactured using the revised, final epoetin content target (Section 3.2) ▪ Measured epoetin content results for all 9 lots within the Epogen/Procrit reference product range; epoetin content results also within the epoetin content range of the Epoetin Hospira DP and Epogen/Procrit lots used in clinical studies ▪ This minor change, though analytically quantifiable, was demonstrated to have no biological impact via functional testing (Section 4.3.6 and Section 11.1)
Manufacturing Process	Addition of commercial product specifications for selected quality attributes and tightening of several proposed specifications	▪ Proposed commercial product specifications were added or tightened per FDA request
Analytical	Sensitivity analyses on analytical assessment of biosimilarity	▪ Multiple sensitivity analyses conducted across the available data sets (i.e., matched replicates within a lot, matched number of lots, matched age of product, random sampling without replacement) ▪ Sensitivity analyses supported the conclusions of the primary analytical similarity assessment
Analytical	Validation of additional methods for use in routine release and stability testing of Epoetin Hospira	▪ Requested methods were optimized and validated, and will be implemented in routine release and stability testing for future manufacturing campaigns

Table 7. Additional Data and Sensitivity Analyses Requested by FDA

Category	Request	How Addressed
Clinical Pharmacology	Sensitivity analyses on PK and PD endpoints including the 90% CI for PD endpoint	▪ Requested sensitivity analyses were conducted (Section 6.1.3 and Section 6.1.4) ▪ Sensitivity analyses supported the PK and PD equivalence conclusions of the pre-specified analyses
Clinical Efficacy	Sensitivity analyses (90% CI for efficacy endpoints, study-level analyses removing data from investigator sites closed for GCP non-compliance)	▪ Requested sensitivity analyses were conducted (Section 6.2.5) ▪ Sensitivity analyses supported the equivalence conclusions of the pre-specified analyses
Clinical Immunogenicity	Updated assay validation with new cutpoints and re-testing of all clinical samples	▪ Radioimmunoprecipitation (RIP) assay and neutralizing anti-recombinant human erythropoietin (anti-rhEPO) assay were updated and validated with more stringent cut-points (Section 6.3.5) ▪ Immunogenicity samples across 6 clinical studies re-tested using the revised cutpoints ▪ Supplemental immunogenicity data analyses supported the conclusions from the original analyses, demonstrating comparable immunogenicity profile between Epoetin Hospira and Epogen

Taken together, the totality of scientific evidence (Figure 6), as summarized in this Briefing Document, establishes the biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product.

Figure 6. Summary of Evidence Demonstrating Biosimilarity



2. INTRODUCTION

2.1. Biosimilar Pathway

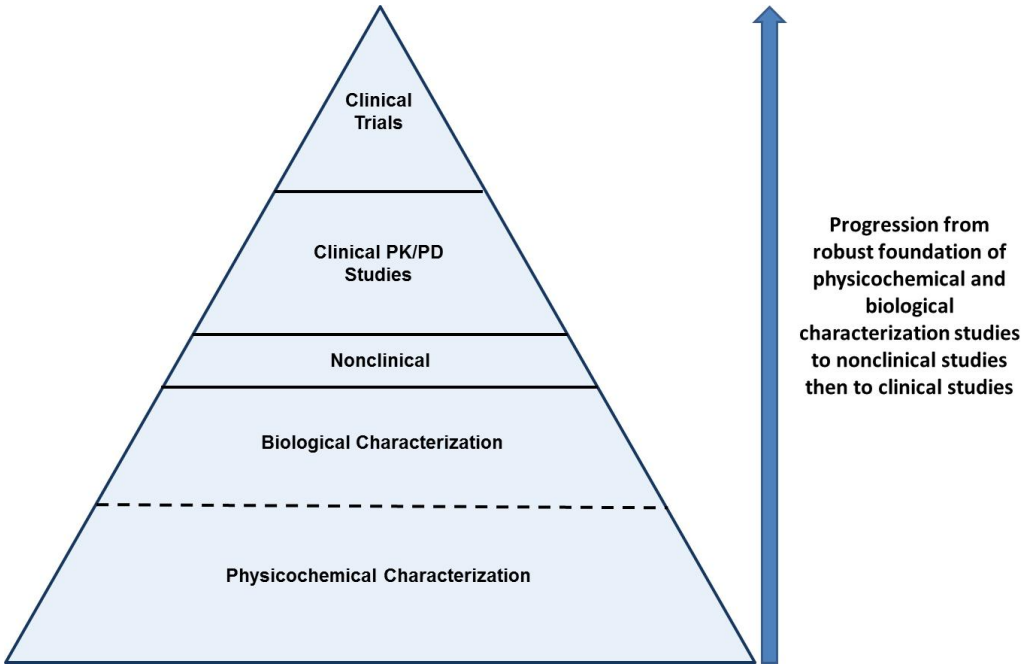
The Biologics Price Competition and Innovation (BPCI) Act of 2009 created an abbreviated licensure pathway for biological products shown to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product (the "reference product").

In the paradigm for development of biosimilar products under section 351(k) of the PHS Act, a proposed biological product that is demonstrated to be biosimilar to a reference product can rely on certain existing scientific knowledge about the safety, purity, and potency of the reference product to be licensed under an abbreviated pathway based on less than a full complement of product-specific nonclinical and clinical data. Specifically, the Act and associated FDA guidance state that a limited number of clinical studies would likely be sufficient for the assessment of "no clinically meaningful differences" between the proposed biological product and the reference product, explaining "if the reference product has a long, relatively safe marketing history and there have been multiple versions of the reference product on the market with no apparent differences in clinical safety and effectiveness profiles, there may be a basis for a selective and targeted approach to the clinical program."

The underlying basis of biosimilarity is that a biological product that is shown to be highly similar to a reference product in structure and function can be expected to perform like the reference product in the clinical setting.

FDA guidance recommends a stepwise approach in generating the data needed to demonstrate biosimilarity. Using this approach, any residual uncertainty about the biosimilarity of the proposed product to the reference product is evaluated and used to inform the following steps, in order to address that uncertainty (Figure 7).

Figure 7. Biosimilarity Development Program



Ultimately, the determination of biosimilarity is based on the "totality of evidence" obtained during both analytical and clinical evaluation, which aims to demonstrates that the proposed biosimilar is "highly similar" with "no clinically meaningful differences" to the reference product.

Where biosimilarity is demonstrated, FDA guidance also allows for extrapolation of data across indications based on sufficient scientific justification. FDA guidance provides recommendations on the approach to scientifically justify extrapolation that should address:

- The mechanism of action(s) in each condition of use for which licensure is sought;
- The PK and biodistribution of the product in different patient populations, and PD measures that may provide important information on the mechanism of action;
- The immunogenicity of the product in different patient populations;
- Differences in expected toxicities in each condition of use and patient population (including whether expected toxicities are related to the pharmacological activity of the product or to off-target activities);
- Any other factor that may affect the safety or effectiveness.

2.2. Erythropoietin Biology and Mechanism of Action

Erythropoietin, a naturally occurring 30,400 Da glycosylated protein, stimulates the proliferation and differentiation of erythroid precursors in the bone marrow.

In the normal physiologic state, erythropoietin operates in a negative feedback loop. Hypoxemia leads to marked upregulation of erythropoietin production by normal kidney cells, stimulating erythropoiesis in a dose-dependent manner and thereby promoting the viability, proliferation, and terminal differentiation of erythroid precursors, ultimately increasing RBC mass.

2.3. Epogen/Procrit

The reference product, Epogen/Procrit, was first approved in the US in 1989 for the "treatment of anemia associated with chronic renal failure, including patients on dialysis (end stage renal disease) and patients not on dialysis" (Epogen PI, 2014). Epogen/Procrit is now approved for the following indications:

- For the treatment of anemia due to CKD, including patients on dialysis and not on dialysis to decrease the need for RBC transfusion;
- For the treatment of anemia due to zidovudine administered at ≤ 4200 mg/week in HIV-infected patients with endogenous serum erythropoietin levels of ≤ 500 mUnits/milliliter (mL);
- For the treatment of anemia in patients with non-myeloid malignancies where anemia is due to the effect of concomitant myelosuppressive chemotherapy, and upon initiation, there is a minimum of 2 additional months of planned chemotherapy;
- To reduce the need for allogeneic RBC transfusions among patients with perioperative Hb > 10 to ≤ 13 g/dL who are at high risk for perioperative blood loss from elective noncardiac, nonvascular surgery.

Epogen/Procrit has been used in clinical practice in the US for over 25 years and has a well-characterized efficacy and safety profile.

2.4. Epoetin Hospira

2.4.1. Product Details

Epoetin Hospira Injection (hereafter referred to as Epoetin Hospira) originated from the development of the Hospira biosimilar product, Retacrit™, approved in the European Union (EU). EU-approved Retacrit is a human recombinant epoetin biosimilar to Eprex® (EU-approved epoetin alfa), with indications for treatment of anemia associated with chronic renal failure or chemotherapy for solid tumors, malignant lymphoma, or multiple myeloma. EU-approved Retacrit was approved in compliance with the European Medicines Agency (EMA) guidelines for the development of biosimilar recombinant erythropoietin and meets the European Pharmacopoeia monograph requirements for erythropoietin. In accordance with these guidelines, biosimilarity of EU-approved Retacrit to the Eprex reference product has been established.

Epoetin Hospira (epoetin alfa) was developed as a proposed biosimilar to US Epogen®/Procrit® (hereafter Epogen/Procrit reference product or Epogen reference product). Additional details regarding the EU Retacrit and Epoetin Hospira manufacturing processes and DP formulations are provided in Section 3.2.

2.4.2. Regulatory History and FDA Interaction

In 2008, the manufacturing process used to produce the EU-approved biosimilar Retacrit was scaled-up and transferred to the US to initiate the Epoetin Hospira US biosimilar development program. As part of this transfer, a new working cell bank was generated from the same cell line and master cell bank used in the EU-Retacrit program. Clinical development of Epoetin Hospira was initiated under a US Investigational New Drug Application (IND) in December 2009, pre-dating the BPCI Act and the biosimilar pathway.

Prior to submission of the Epoetin Hospira Biologics License Application (BLA), a number of meetings were held with FDA to discuss and gain concurrence on the approach for demonstration of biosimilarity across the totality of the analytical, nonclinical and clinical data package. The Epoetin Hospira program advanced through product development based on the availability of draft FDA biosimilar guidance documents and in accordance with evolving FDA expectations as discussed at meetings and in ongoing interactions with FDA.

The clinical studies for Epoetin Hospira were exclusively conducted in the US under the IND. Study protocols were submitted to FDA for review, and Agency feedback was incorporated into the final protocols prior to study conduct. FDA advice was also solicited and incorporated into the comparative clinical studies including Statistical Analysis Plans (SAPs) and the integrated analyses plans for efficacy and safety as well as the Pediatric Study Plan to support the BLA.

Following submission of the BLA, Pfizer addressed information requests and a Complete Response (CR) letter from FDA requesting additional data and sensitivity analyses across the totality of evidence to align with the most current FDA expectations and to ensure the robustness of the data demonstrating biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product.

2.4.3. Overview of the Epoetin Hospira Development Program

The Epoetin Hospira biosimilar development program includes comprehensive analytical, nonclinical, and clinical studies, as recommended by the FDA, to establish biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product (Figure 8). Comparative clinical studies were conducted in healthy volunteers and in CKD patients on hemodialysis. A scientific justification for extrapolation to all other indications was provided in the BLA. A biosimilar interchangeability designation is not being sought with this BLA.

The foundation of the Epoetin Hospira biosimilarity assessment was extensive physicochemical and functional analytical characterization of both Epoetin Hospira and the Epogen/Procrit reference product. In total, 35 Epoetin Hospira Drug Product (DP) lots, 9 Epoetin Hospira DS lots, and 54 reference product lots were evaluated.

Two GLP-compliant 13-week repeat-dose comparative toxicity studies (including PD, PK/TK, and immunogenicity evaluations) were conducted – one with subcutaneous administration in rats and one with intravenous administration in dogs. The clinical development program for Epoetin Hospira comprised seven clinical studies: three PK/PD studies and four clinical efficacy and safety studies (Table 8).

Figure 8. Overview of Sources of Epoetin Hospira Biosimilarity Data

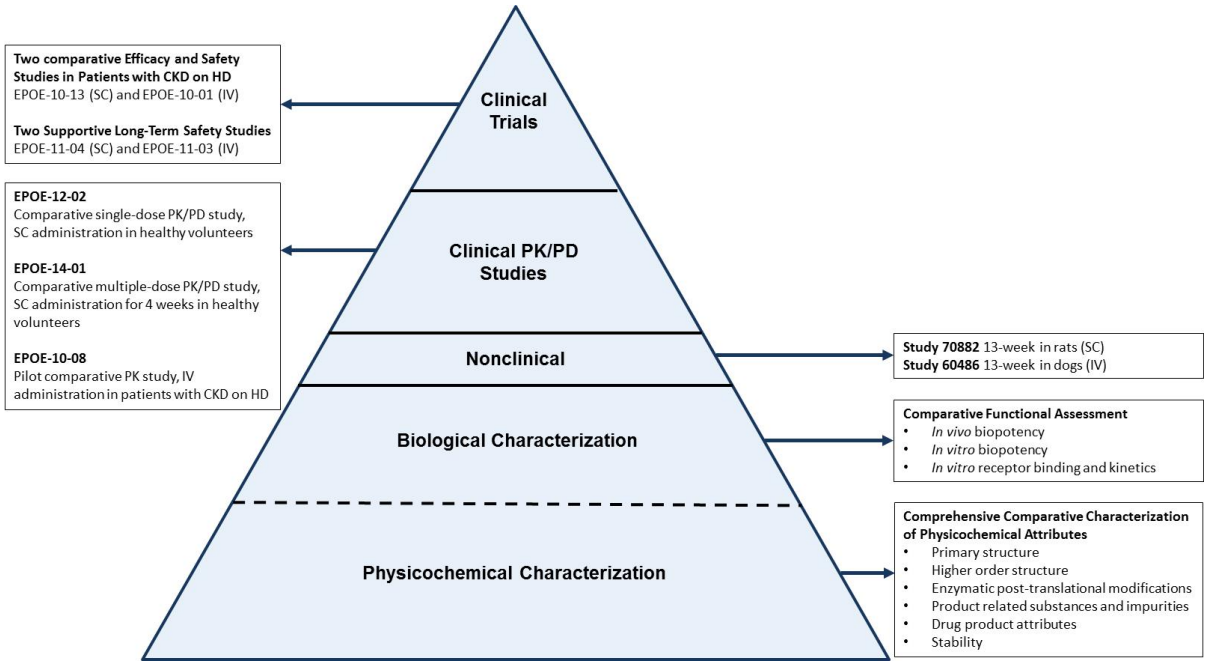


Table 8. Clinical Studies in the Epoetin Hospira Clinical Development Program

Study*	Description	Route of Administration	Type/Number of Subjects
PK/PD Studies			
EPOE-12-02	Comparative single-dose PK/PD study	Subcutaneous	HS, 81 randomized
EPOE-14-01	Comparative multiple-dose PD/PK study	Subcutaneous	HS, 129 randomized
EPOE-10-08*	Pilot comparative PK study	Intravenous	CKD on HD; 105 randomized
Clinical Efficacy and Safety Studies			
EPOE-10-13	Comparative safety and efficacy study	Subcutaneous	CKD on HD; 320 randomized
EPOE-10-01	Comparative safety and efficacy study	Intravenous	CKD on HD; 612 randomized
EPOE-11-04	Supportive long-term safety study	Subcutaneous	CKD on HD; 173 enrolled
EPOE-11-03	Supportive long-term safety study	Intravenous	CKD on HD; 414 enrolled

CKD, chronic kidney disease; HD, hemodialysis; HS, healthy subjects.
*Note: All studies, except EPOE-10-08, used the same late stage development formulation. EPOE-10-08 was a pilot PK study using an early formulation.

Pfizer followed FDA's stepwise approach in generating the data to demonstrate biosimilarity of Epoetin Hospira to Epogen/Procrit. Consistent with this approach, any residual uncertainty due to minor differences observed between Epoetin Hospira and Epogen/Procrit at any stage was evaluated and informed the following steps so as to address that uncertainty. A brief summary of the minor differences observed and how these potential residual uncertainties were addressed across the totality of evidence in the BLA is provided in Table 9. Ultimately, the analytical, nonclinical and clinical evaluation demonstrates that Epoetin Hospira is "highly similar" with "no clinically meaningful differences" to the Epogen/Procrit reference product.

Table 9. Minor Differences Observed between Epoetin Hospira and Epogen/Procrit and How Addressed

Category	Observed Difference	Potential Impact	How Addressed
Analytical - Physicochemical Structure	Relative abundance of some N- and O-glycan structures (Section 4.3.3)	PK/PD	▪ Functional testing using a mouse PD model demonstrates no PD impact ▪ Equivalence demonstrated in single-dose (EPOE-12-02) and multiple dose (EPOE-14-01) PK/PD studies
Analytical - Physicochemical Structure	Cys29-Cys33 trisulfide (Table 13)	Biopotency Immunogenicity	▪ Functional testing results demonstrate no impact on *in vitro* specific activity and *in vivo* biopotency ▪ No differences observed in immunogenicity in clinical studies
Analytical - Physicochemical Structure	Deamidated Asn147 (Section 4.3.4.2)	Biopotency Immunogenicity	▪ Functional testing results demonstrate no impact on *in vitro* specific activity and *in vivo* biopotency ▪ No differences observed in immunogenicity in clinical studies
Nonclinical - Comparative 13-week Rat SC Toxicity Study	PD and PK/TK (Section 5)	PK/PD Immunogenicity	▪ Equivalence demonstrated in single-dose (EPOE-12-02) and multiple dose (EPOE-14-01) PK/PD studies ▪ No differences observed in immunogenicity in clinical studies

As noted in the previous section, Pfizer also addressed FDA information requests and a Complete Response (CR) letter issued by FDA as part of the review of the Epoetin Hospira BLA. FDA requested additional data and sensitivity analyses to align with the most current FDA expectations and to ensure the robustness of the data demonstrating biosimilarity of Epoetin Hospira to the Epogen/Procrit reference product. A high-level summary of the

additional data and analyses in the BLA is provided in Table 10. These sensitivity analyses are consistent with the pre-specified analyses and further support the conclusion that biosimilarity has been demonstrated between Epoetin Hospira and the Epogen/Procrit reference product.

Table 10. Additional Data and Sensitivity Analyses Requested by FDA

Category	Request	How Addressed
Manufacturing Process	Minor adjustment of the Epoetin Hospira Drug Product (DP) manufacturing process content target to more closely match epoetin protein content of the reference product	▪ Revised target implemented during the BLA review; nine (9) lots of Epoetin Hospira DP (three lots at each of the 2000, 10,000 and 40,000 U/mL dose strengths) were manufactured using the revised, final epoetin content target (Section 3.2) ▪ Measured epoetin content results for all 9 lots within the Epogen/Procrit reference product range; epoetin content results also within the epoetin content range of the Epoetin Hospira DP and Epogen/Procrit lots used in clinical studies ▪ This minor change, though analytically quantifiable, was demonstrated to have no biological impact via functional testing (Section 4.3.6 and Section 11.1)
Manufacturing Process	Addition of commercial product specifications for selected quality attributes and tightening of several proposed specifications	▪ Proposed commercial product specifications were added or tightened per FDA request
Analytical	Sensitivity analyses on analytical assessment of biosimilarity	▪ Multiple sensitivity analyses conducted across the available data sets (i.e., matched replicates within a lot, matched number of lots, matched age of product, random sampling without replacement) ▪ Sensitivity analyses supported the conclusions of the primary analytical similarity assessment
Analytical	Validation of additional methods for use in routine release and stability testing of Epoetin Hospira	▪ Requested methods were optimized and validated, and will be implemented in routine release and stability testing for future manufacturing campaigns

Table 10. Additional Data and Sensitivity Analyses Requested by FDA

Category	Request	How Addressed
Clinical Pharmacology	Sensitivity analyses on PK and PD endpoints including the 90% CI for PD endpoint	▪ Requested sensitivity analyses were conducted (Section 6.1.3 and Section 6.1.4) ▪ Sensitivity analyses supported the PK and PD equivalence conclusions of the pre-specified analyses
Clinical Efficacy	Sensitivity analyses (90% CI for efficacy endpoints, study-level analyses removing data from investigator sites closed for GCP non-compliance)	▪ Requested sensitivity analyses were conducted (Section 6.2.5) ▪ Sensitivity analyses supported the equivalence conclusions of the pre-specified analyses
Clinical Immunogenicity	Updated assay validation with new cutpoints and re-testing of all clinical samples	▪ Radioimmunoprecipitation (RIP) assay and neutralizing anti-recombinant human erythropoietin (anti-rhEPO) assay were updated and validated with more stringent cut-points (Section 6.3.5) ▪ Immunogenicity samples across 6 clinical studies re-tested using the revised cutpoints ▪ Supplemental immunogenicity data analyses supported the conclusions from the original analyses, demonstrating comparable immunogenicity profile between Epoetin Hospira and Epogen

The Epoetin Hospira data package meets all of the regulatory requirements for biosimilarity and provides the totality of evidence to demonstrate that Epoetin Hospira is “highly similar” with “no clinically meaningful differences” to the Epogen/Procrit reference product.

3. EPOETIN HOSPIRA MANUFACTURING OVERVIEW

3.1. Drug Substance Process Overview

Epoetin Hospira originated from the development of Pfizer's EU-approved biosimilar Retacrit. The engineered Chinese hamster ovary (CHO) cell line used to manufacture the Epoetin Hospira Drug Substance (DS) is the same as that used to produce the EU Retacrit DS. The DS manufacturing process for EU Retacrit was transferred to a larger scale manufacturing facility in the U.S. and was validated to support the Epoetin Hospira program. The cell culture, harvest and purification steps used to manufacture the Epoetin Hospira DS are the same as those for EU Retacrit with minor modifications to enable production at a larger scale. Analytical testing was conducted to compare the Epoetin Hospira and EU Retacrit DS and Drug Product (DP). The comparative data, included in the BLA, demonstrate that the structural and functional attributes of EU Retacrit and Epoetin Hospira are comparable.

All of the comparative clinical studies, with the exception of the pilot PK study (EPOE-10-08), were conducted with Epoetin Hospira DP manufactured from DS that was produced using the validated process at the commercial manufacturing site and scale.

3.2. Drug Product Overview

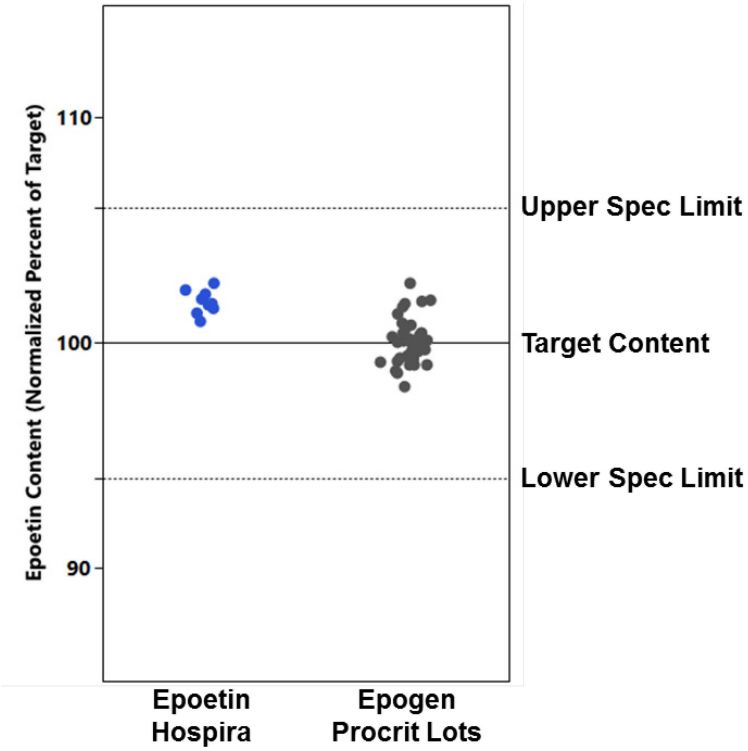
Epoetin Hospira DP is manufactured in dose strengths of 2000, 3000, 4000, 10,000, and 40,000 U/mL. These strengths are identical to the marketed strengths for the Epogen/Procrit reference product. The Epoetin Hospira DP formulation is the same as the EU Retacrit DP formulation and contains excipients and stabilizers that are commonly used in modern biopharmaceutical product formulations to avoid the use of human-sourced raw materials. Amino acid excipients and a polysorbate stabilizer are used in place of the plasma-derived human serum albumin (HSA) protein stabilizer present in the Epogen and Procrit formulations. The differences in inactive formulation components relative to the reference product were demonstrated not to impact the results of the analytical, nonclinical and clinical similarity of Epoetin Hospira to the Epogen/Procrit reference product. The stability of the Epoetin Hospira DP was demonstrated under long-term and accelerated storage conditions and the proposed product shelf-life is supported by an extensive stability data set provided in the BLA.

A defined epoetin content target, expressed in micrograms of epoetin per milliliter of DP solution (µg/mL), was established to match the epoetin content of the Epogen/Procrit reference product. A minor revision to the Epoetin Hospira DP content target, representing a shift in the epoetin content target of approximately 3.5%, was requested by FDA during the BLA review. The revised target was implemented to enhance the similarity to the Epogen/Procrit reference product. Nine lots of Epoetin Hospira DP (three lots at each of the 2000, 10,000 and 40,000 U/mL dose strengths) were manufactured using the revised, final epoetin content target, all of which have a measured epoetin content that falls within the Epogen/Procrit reference product range. The epoetin content results for the nine lots are also within the epoetin content range of the Epoetin Hospira DP and Epogen/Procrit lots used in

clinical studies. In addition, this minor change, though analytically quantifiable, was demonstrated to have no biological impact via functional testing, as highlighted in Section 4.3.6 and Section 11.1.

The epoetin content results for the nine Epoetin Hospira DP lots and the Epogen/Procrit lots used in the analytical studies are shown relative to the proposed commercial epoetin content specifications in Figure 9. The difference in epoetin content between the target value and the mean value for the nine Epoetin Hospira DP lots is small (approximately 1.8%). The Epoetin Hospira results are all within both the product specification and the Epogen/Procrit reference product range. The Epoetin Hospira specifications were established based on the mean epoetin content for the Epogen/Procrit reference product. These limits ensure that all lots of Epoetin Hospira DP released to the market have an epoetin content consistent with that of the Epogen/Procrit reference product. All commercial Epoetin Hospira DP lots must meet the content specifications at release and throughout the proposed shelf-life.

Figure 9. Epoetin Content Specifications for Epoetin Hospira Lots



4. ANALYTICAL ASSESSMENT OF BIOSIMILARITY FOR EPOETIN HOSPIRA AND THE EPOGEN/PROCRIT REFERENCE PRODUCT

Epoetin Hospira was developed to be highly similar to the Epogen/Procrit reference product. Both products are expressed in Chinese hamster ovary (CHO) cells engineered to produce the epoetin protein. However, given the proprietary nature of manufacturing cell lines and processes, minor differences in the relative amounts of a small number of product attributes are expected between Epoetin Hospira and Epogen/Procrit reference product. One aim of the analytical assessment of biosimilarity was to comparatively evaluate the physicochemical properties of Epoetin Hospira and the Epogen/Procrit reference product to ascertain if any such differences exist between the two products. A subsequent aim of the analytical assessment was to determine if the functional activity of Epoetin Hospira was impacted by any such differences.

The results of the analytical similarity assessment demonstrate that Epoetin Hospira is highly similar to the US-licensed Epogen/Procrit reference product with no analytical differences that impact functional activity.

4.1. Summary of Analytical Evidence

Comprehensive analytical studies were conducted as part of the overall Epoetin Hospira Biosimilarity Assessment. These studies included structural and functional characterization using multiple orthogonal analytical methods to evaluate:

- Primary structure
- Higher order structure
- Post-translational modifications
- Product-related substances and impurities
- Drug product attributes
- Functional activity
- Accelerated stability and forced degradation

The analytical methods used for the similarity assessment include validated methods used for routine lot release and stability testing of the Epoetin Hospira product and qualified methods used for extended characterization. The characterization methods were developed and implemented to support a comprehensive, comparative characterization of Epoetin Hospira and the Epogen/Procrit reference product.

Comparative testing was completed using multiple lots of Epoetin Hospira and the Epogen/Procrit reference product across the product shelf-lives including:

- 9 commercial-scale lots of Epoetin Hospira Drug Substance manufactured between July 2009 and December 2013;

- 35 commercial-scale lots of Epoetin Hospira Drug Product (including clinical lots) manufactured between August 2011 and July 2015; and
- 42 lots of the Epogen (representing the 2000, 3000, 4000, and 10,000 U/mL dose strengths) and 12 lots of Procrit (representing the 40,000 U/mL dose strength) produced over a greater than two year manufacturing period and representing 15 months of shelf-life.

4.2. Assessment of Attribute Criticality and Statistical Tier Assignments

Pfizer completed a comprehensive Critical Quality Attribute (CQA) assessment to evaluate more than 80 Epoetin Hopsira DS and DP attributes in terms of their potential impact on patient safety (toxicology, immunogenicity) and clinical performance (activity, PK/PD and efficacy). The CQA assessment was based on a review of available scientific literature and supporting studies conducted by Pfizer using samples with enriched variants and/or impurities. The CQAs were used to inform the manufacturing control strategies for Epoetin Hospira DS and DP and the analytical similarity assessment. The CQA assessment enabled the designation of attributes as high-, medium-, and low-criticality. High- and medium-criticality attributes are those with an established or potential link to patient safety and/or clinical performance, respectively. Low-criticality attributes do not impact patient safety or clinical performance and are designated as non-CQAs. Listings of the high-, medium- and low-criticality attributes are provided in Table 11, Table 12 and Table 13, respectively.

Statistical analyses were used as a component of the analytical similarity assessment to support quantitative comparisons of the analytical results for Epoetin Hospira and the Epogen/Procrit reference product. The statistical analyses were aligned with the tiered construct proposed by FDA in which approaches of varying statistical rigor are used to compare product attributes. Three statistical analysis tiers (Tiers 1, 2 and 3) were defined, with the highest degree of statistical rigor applied to Tier 1 attributes.

Tier 1 attributes are the most important attributes and are linked to the clinically relevant mechanism of action of epoetin. In consultation with FDA, Pfizer assigned two product attributes, *In Vivo* Biopotency and *In Vitro* Specific Activity, to Tier 1. Equivalence testing was performed for Tier 1 attributes using equivalence margins of ± 1.5 SD_{ref} as defined by FDA, where SD_{ref} is the standard deviation of the reference product results. Analytical equivalence was demonstrated when the constructed 90% confidence interval (CI) around the mean difference between the Epoetin Hospira and Epogen/Procrit reference product lots fell within the upper and lower equivalence margins.

Tier 2 attributes are high-criticality attributes for which the direct link to the epoetin mechanism of action is less certain or that are redundant relative to Tier 1 attributes. These include attributes linked to *in vivo* half-life, important structural motifs, product strength attributes, and selected product related substances and impurities. Tier 2 attributes were assessed using the quality range approach recommended by the FDA. The quality range is defined as the mean $_{ref}$ ±3 SD, where SD is the standard deviation of the reference product results. This $mean_{ref}$ ± 3SD range theoretically captures 99.7% of the results from reference

product testing, assuming a normal distribution. Therefore the quality range of mean $_{ref}$ ±3 SD is representative of the variability expected in the Epogen/Procrit reference product.

Tier 3 attributes are low-criticality attributes, those for which the data are not amenable to formal statistical comparisons, or attributes that are redundant to Tier 2 attributes. Tier 3 attributes were compared qualitatively using graphical comparisons or other qualitative approaches.

4.3. Analytical Similarity Results

4.3.1. Analytical Similarity Results Summary

A summary of the physicochemical and functional assay results from the analytical similarity studies comparing Epoetin Hospira to the Epogen/Procrit reference product is provided in Table 11, Table 12, and Table 13. Results for the high-criticality attributes that are directly linked to the epoetin *in vivo* mechanism of action and product safety/efficacy are shown in Table 11. Results for the medium and low criticality attributes that are not directly linked to the mechanism of action or product safety/efficacy are shown in Table 12 and Table 13.

Table 11. Summary of Analytical Similarity Assessment Results for Attributes with High Criticality

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Assessment Criteria	Results	Analytical Similarity Demonstrated
Primary Structure	Amino Acid Sequence by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	Same amino acid sequence as Epogen/Procrit	Identical amino acid sequence	✓
	Disulfide Mapping by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	Same sites as Epogen/Procrit	Same disulfide linkages	✓
	Sites of N-Linked Glycosylation by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	Same sites as Epogen/Procrit	Same sites of glycosylation	✓
	Molecular weight of de-glycosylated epoetin by LC-MS	Qualitative comparison	Molecular weight consistent with amino acid sequence of Epogen/Procrit	Measured molecular weight within 0.0 Da of Epogen/ Procrit molecular weight	✓
Higher Order Structure	Secondary structure by Far-UV CD: α-helix, β-structure, Random Coil	Tier 2 – Quality range	α-helix: 56 – 67% Total β-structure: 18 – 25% Random coil: 16 – 21%	α-helix: 57 – 66% Total β-structure: 18 – 24% Random coil: 16 – 20% (Figure 12)	✓
	Secondary structure by FTIR: Spectral Comparability Index	Tier 3 – Qualitative comparison	N/A	Visually similar spectra[b] (Figure 13)	✓
	Tertiary structure by Intrinsic Fluorescence: Fluorescence Emission Maximum (λ_{max})	Tier 3 – Qualitative comparison	N/A	Visually similar[b] and similar λ_{max}	✓

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Assessment Criteria	Results	Analytical Similarity Demonstrated
Higher Order Structure (cont'd)	Tertiary structure by Near-UV CD: Peak maxima for Phe, Tyr, Trp	Tier 3 – Qualitative comparison	N/A	Visually similar spectra[b] (Figure 15)	✓
	Melting temperature (T_m) by DSC	Tier 2 – Quality range	58.5 – 59.9°C	58.6 – 59.3°C (Figure 14)	✓
	Hydrodynamic Properties and Molecular Weight by SV-AUC	Qualitative comparison	N/A	Similar s-value and molecular weight	✓
Enzymatic Post-Translational Modifications	Total Sialic Acids by RP-HPLC	Tier 2 – Quality range	292 – 494 nmol/mg of epoetin	352 – 460 nmol/mg of epoetin	✓
	N-glycolylneuraminic acid (NeuGc)[c] by RP-HPLC	Tier 3 – Qualitative comparison	N/A	Lower levels of non-human NeuGc sialic acid species in Epoetin Hospira	✓
	α-Gal-1,3-Gal by HPAEC-PAD	Tier 3 – Qualitative comparison	N/A	Similar	✓
Product Related Substances and Impurities	Deamidation at Asn 147 by Trypsin Peptide Map (RP-UPLC-MS)	Tier 2 – Quality range	0.3 – 0.7 %	0.4 – 1.5 %	Minor quantitative differences (with no impact on biopotency[d])
	Trisulfide (T2=T20 Trisulfide at Cys7-Cys161) by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	N/A	Similar	✓
	Dimer and Other High Molecular Weight Species (HMWS) by Quantitative Western Blot	Tier 3 – Qualitative comparison	N/A	Levels of HMWS consistent with levels in Epogen/Procrit	✓

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Assessment Criteria	Results	Analytical Similarity Demonstrated
Drug Product Attributes	Epoetin Content by RP-UPLC	Tier 2 – Quality range	7.9 – 8.7 µg per 1000 Units	8.4 – 8.5 µg per 1000 Units[e]	✓
	Container Fill Volume	Tier 2 – Quality range	1.085 – 1.125 mL	1.087 – 1.109 mL	✓
	Container Deliverable Volume	Tier 2 – Quality range	1.030 – 1.097 mL	1.041 – 1.064 mL	✓
	Particulate Matter by MFI (≥ 25 µm, ≥ 10 µm, ≥ 5 µm and ≥ 2 µm)	Qualitative comparison	N/A	Levels 5- to 10-folder lower in Epoetin Hospira	✓
	Particulate Matter by NanoSight (0.1 – 1 µm)	Qualitative comparison	N/A	Levels significantly lower in Epoetin Hospira	
Functional Attributes	*In Vivo* Biopotency (Normocythaemic mouse)	Tier 1 – Equivalence Testing	-11.024 – 11.024% (Equivalence Margins)	-7.503 – 1.366% (Lower and Upper 90% CI)	✓
	In Vitro Specific Activity	Tier 1 – Equivalence Testing	-5.602 – 5.602 Units/µg (Equivalence Margins)	2.023 – 5.131 Units/µg (Lower and Upper 90% CI)	✓
	In Vivo Specific Activity	Tier 2 – Quality range	92 – 148 Units/µg	106 – 128 Units/µg	✓
	Total *In Vivo* Biopotency per Container	Tier 2 – Quality range	82 – 129 % × mL	94 – 111% × mL	✓
	Total *In Vitro* Biopotency per Container	Tier 2 – Quality range	95 – 114 % × mL	104 – 114% × mL	✓
	Competitive Receptor Binding	Tier 2 – Quality range	84 – 113%	92 – 107%	✓

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach[a]	Assessment Criteria	Results	Analytical Similarity Demonstrated
Functional Attributes (cont'd)	Receptor Binding Affinity and Kinetics by Surface Plasmon Resonance (K_D, k_{on} and k_{off})	Tier 3 – Qualitative comparison	N/A	SPR response curves are comparable and calculated K_D, k_{on} and k_{off} values are consistent with Epogen/Procrit	✓

CD, circular dichroism; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; HMWS, high molecular weight species; HPAEC-PAD, high performance anion exchange chromatography with pulsed amperometric detection; LC-MS, liquid chromatography mass spectrometry; MFI, micro-flow imaging; RP-UPLC, reversed phase ultra performance liquid chromatography; RP-UPLC-MS, reversed phase ultra performance liquid chromatography mass spectrometry; UV, ultraviolet

[a] Quality ranges for Tier 2 attributes represent ±3SD of the reference product mean. The equivalence margin for Tier 1 attributes is ±1.5SD of the reference product mean.

[b] Visually similar: Similar spectral features, including position and magnitude of spectral minima and/or maxima. No new peaks or bands greater than the limit of detection of the method.

[c] N-glycolylneuraminic acid (NeuGc) is a non-human sialic species that may be present at low levels in sialylated glycoproteins expressed in non-human cell lines

[d] *In vitro* testing demonstrates that there is no correlation between the amount of Asn 147 deamidation and epoetin biological activity at the low levels observed in the Epoetin Hospira product (1.5% or less), as described in Section 4.3.4.2. The minor difference in levels of Asn 147 deamidation observed between Epoetin Hospira nd the Epogen/Procrit reference product therefore does not represent a biologically meaningful difference.

[e] Results for the Epoetin Hospira lots manufactured following the epoetin content target change described in Section 3.2.

Table 12. Summary of Analytical Similarity Assessment Results for Attributes with Medium Criticality

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach	Assessment Criteria	Results	Analytical Similarity Demonstrated
Primary Structure	Free Cysteine residues by RP-HPLC	Qualitative comparison	N/A	Levels below the limit of detection[a]	✓
Enzymatic Post-Translational Modifications	N-Linked Glycans: Sialic Acid Distribution (Di-, Tri, Tetra-Sialylated) by Anion Exchange HPLC	Tier 3 – Qualitative comparison	N/A	Same sialylated glycan structures as Epogen/Procrit	Same structures observed with minor quantitative differences[b]
	N-Linked Glycans: Di-, Tri- and Tetra-Antennary Structures by HILIC-UPLC-FLD	Tier 3 – Qualitative comparison	N/A	Same antennary glycan structures as Epogen/Procrit	

HILIC-UPLC-FLD, hydrophobic interaction ultra performance liquid chromatography with fluorescence detection; HPLC, high performance liquid chromatography; RP-HPLC, reversed phase high performance liquid chromatography

[a] The observed low levels of free sulfhydryls demonstrate that the disulfide bonds are completely formed as expected for properly folded epoetin.
[b] Differences in the relative abundance of glycan structures were evaluated extensively using *in vitro* bioassays and an *in vivo* mouse PD model (normocythaemic mouse). These *in vitro* and *in vivo* studies demonstrated conclusively that any quantitative differences observed in the epoetin glycan profile do not impact the binding affinity or binding kinetics of Epoetin Hospira to the epoetin receptor or the *in vivo* half-life and biological activity of Epoetin Hospira relative to the Epogen/Procrit reference product. These *in vitro* and *in vivo* studies are also supported by the comparative nonclinical and clinical study results described in Section 5 and Section 6.

Table 13. Summary of Analytical Similarity Assessment Results for Attributes with Low Criticality

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach	Assessment Criteria	Results	Analytical Similarity Demonstrated
Primary Structure	Sites of O-Linked Glycosylation by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	Sites must be the same as those observed for Epogen/Procrit	Same sites of glycosylation	✓
Enzymatic Post-Translational Modifications	N-Linked Glycans: N-acetyllactosamine (Lac) repeats by HILIC-UPLC-FLD	Tier 3 – Qualitative comparison	N/A	Same glycan structures containing Lac repeats with minor quantitative differences	Same structures observed with minor quantitative differences[a,b]
	O-Linked Glycan Profile by T13 Peptide LC-MS	Tier 3 – Qualitative comparison	N/A	Same glycan structures with minor quantitative differences	
	Isoform distribution by CZE	Tier 3 – Qualitative comparison	N/A	Same isoforms with some quantitative differences in distribution (linked to differences in O-linked glycan profile and N-linked glycan Lac repeats)	Same isoform profile with some quantitative differences
Product Related Substances and Impurities	Oxidation at Met 54 by Lys-C, K4 Peptide Map	Tier 3 – Qualitative comparison	N/A	Similar	✓
	Oxidation at Trp 64 and Trp 88 by Lys-C, K4 Peptide Map	Qualitative comparison	N/A	Similar	✓
	Deamidation at Gln 86 by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	N/A	Similar	✓

Attribute Category	Attribute Measured	Tier or Similarity Assessment Approach	Assessment Criteria	Results	Analytical Similarity Demonstrated
Product Related Substances and Impurities (cont'd)	Aspartic acid isomerization at Asp 123 by Trypsin Peptide Map (RP-UPLC-MS)	Tier 3 – Qualitative comparison	N/A	Similar	✓
	Aspartic acid isomerization at Asp 43 by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	N/A	Similar	✓
	Trisulfide (T5 Trisulfide at [Cys29-Cys33]) by Trypsin Peptide Map (RP-UPLC-MS)	Tier 3 – Qualitative comparison	N/A	Same trisulfide species observed in Epogen/Procrit reference product	Higher levels observed in Epoetin Hospira; no impact on biopotency [c]
	Oxidation at Trp 51 by Trypsin Peptide Map (RP-UPLC-MS)	Qualitative comparison	N/A	Similar	✓

CZE, capillary zone electrophoresis; HILIC-UPLC-FLD, hydrophobic interaction ultra performance liquid chromatography with fluorescence detection; RP-UPLC-MS, reversed phase ultra performance liquid chromatography mass spectrometry,

[a] Minor differences in the relative abundance of glycan structures were evaluated extensively using *in vitro* bioassays and an *in vivo* mouse PD model (normocythaemic mouse). These *in vitro* and *in vivo* studies demonstrated conclusively that any quantitative differences observed in the epoetin glycan profile do not impact the binding affinity or binding kinetics of Epoetin Hospira to the epoetin receptor or the *in vivo* half-life and biological activity of Epoetin Hospira relative to the Epogen/Procrit reference product. These *in vitro* and *in vivo* studies are also supported by the comparative nonclinical and clinical study results described in Section 5 and Section 6, which utilized Epoetin Hospira and Epogen lots having these minor differences.

[b] O-linked glycans have been demonstrated to be non-critical for epoetin *in vivo* biopotency and half-life and epoetin missing the O-linked glycans is fully active *in vivo* (Wasley et al., 1991).

[c] The T5 Trisulfide contains an additional sulfur atom between the two Cys amino acid residues that make up the disulfide bond between amino acid residues Cys29 and Cys33 (R-S-**S**-S-R). This disulfide bond forms a small loop connecting helices A and B of the four-helix bundle. This disulfide bond has been shown not to be critical for maintaining epoetin higher order structure or receptor binding and biopotency. The addition of a sulfur atom within this disulfide bond does not disrupt the epoetin structure.

The comprehensive analytical similarity results, summarized in Table 11, Table 12, and Table 13, demonstrate that Epoetin Hospira is highly similar to the US-licensed reference product, Epogen/Procrit. Epoetin Hospira has an identical amino acid sequence and the same disulfide linkages and sites of O- and N-linked glycosylation as human erythropoietin and the Epogen/Procrit reference product. The similarity of other analytical attributes was confirmed through a rigorous, statistically-based comparative analysis that concluded no biologically meaningful differences exist between Epoetin Hospira and Epogen/Procrit. Detailed analytical results summaries are provided for selected high-criticality attributes in Section 4.3.2, Section 4.3.3, Section 4.3.4, Section 4.3.5, and Section 4.3.6.

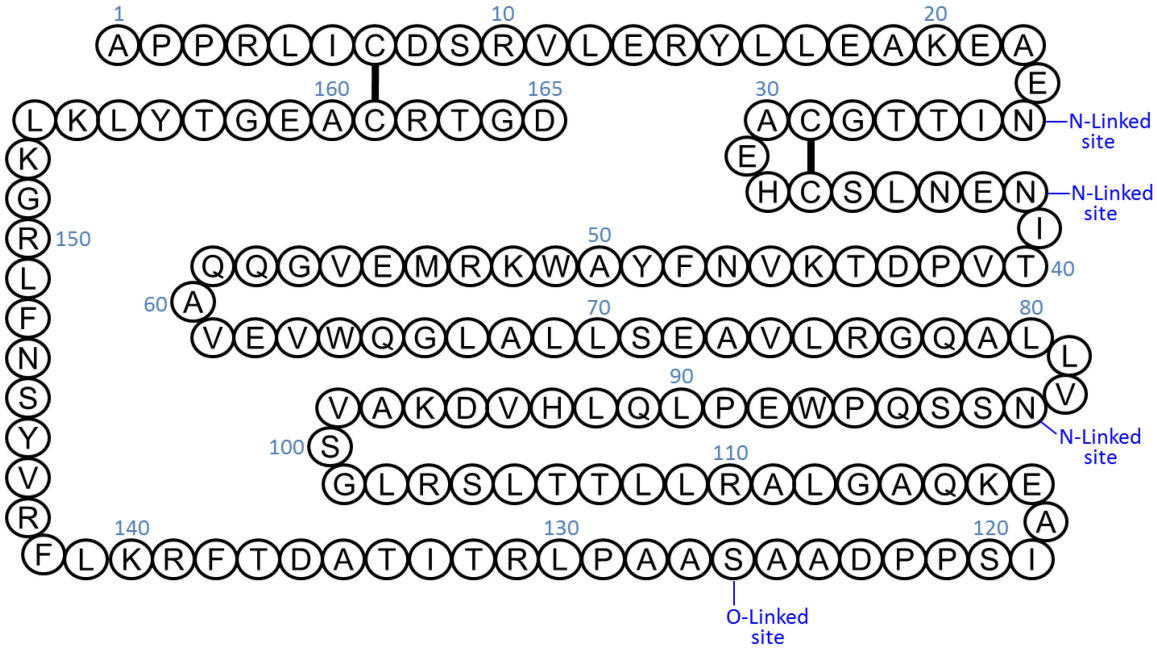
4.3.2. Primary and Higher Order Structure

4.3.2.1. Primary Structure

Epoetin is a member of a family of cytokines that fold into a four-helix bundle motif (Cheetham et al., 1998). The epoetin sequence contains 165 amino acids including four cysteine (Cys or C) residues that form two intramolecular disulfide bonds between Cys 7-Cys 161 and Cys 29-Cys 33 (Figure 10). The Cys 7-Cys 161 disulfide bridge links the N- and C-termini of the epoetin protein and the Cys 29-Cys 33 disulfide forms a small loop within the region between helices A and B of the four-helix bundle.

The epoetin sequence also contains three N-linked glycosylation sites at amino acid residues asparagine (Asn or N) 24, Asn 38, and Asn 83 and one O-linked glycosylation site at residue serine (Ser or S) 126. The epoetin primary structure refers to the amino acid sequence of the epoetin protein, including the Cys amino acid residues involved in disulfide bonding and the Asn and Ser amino acid residues that are the sites of N- and O-linked glycosylation, respectively.

Figure 10. Schematic of Epoetin Amino Acid Sequence

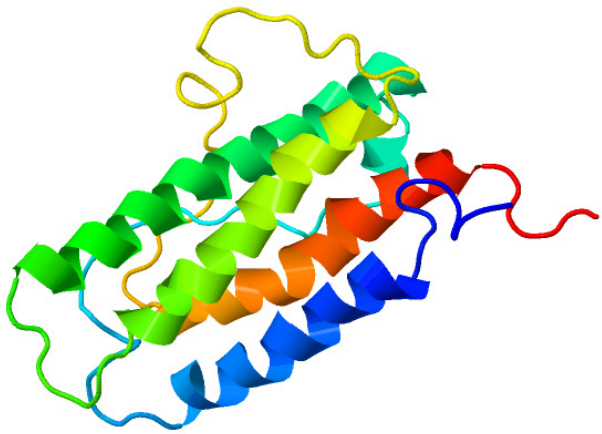


The amino acid sequence of the epoetin present in Epoetin Hospira and the Epogen/Procrit reference product was confirmed by peptide mapping. Peptide mapping was also used to confirm the sites of N- and O-linked glycosylation and the presence of the expected disulfide bonds between Cys 7-Cys 161 and Cys 29-Cys 33. Comparative analysis of the primary structure of the epoetin present in Epoetin Hospira and the Epogen/Procrit reference product demonstrate that the amino acid sequence of the epoetin protein in Epoetin Hospira is identical to that of the Epogen/Procrit reference product. The peptide mapping results also confirm that the sites of N- and O-linked glycosylation and the disulfide linkages are identical for Epoetin Hospira and the Epogen/Procrit reference product.

4.3.2.2. Higher Order Structure

Proper epoetin folding is required for biological activity. Proper epoetin folding includes the formation of key secondary structural elements, including the four α-helices that comprise the four-helix bundle. Subsequent folding of these secondary structural elements forms the tertiary structure of the protein. Structural evaluation of secondary and tertiary structure is an essential component of the analytical similarity assessment and is required to confirm that the epoetin present in Epoetin Hospira is folded in a manner similar to the epoetin present in the Epogen/Procrit reference product (Figure 11).

Figure 11. Structure of Epoetin

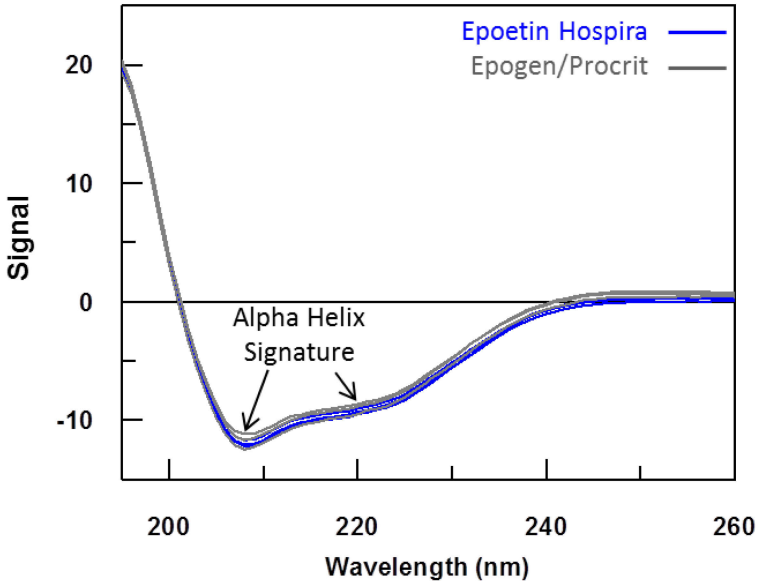


Source: http://www.rcsb.org/pdb/explore/jmol.do?structureId=1BUY)

The higher order structure of the epoetin in Epoetin Hospira and the Epogen/Procrit reference product was evaluated using multiple, orthogonal methods. The relative percentages of α-helix, β-structure, and random coil secondary structures were assessed using Far Ultraviolet Circular Dichroism (Far-UV CD). The Far-UV CD spectra for Epoetin Hospira and the Epogen/Procrit reference product shown in Figure 12 have the characteristic α-helix features and are essentially indistinguishable.

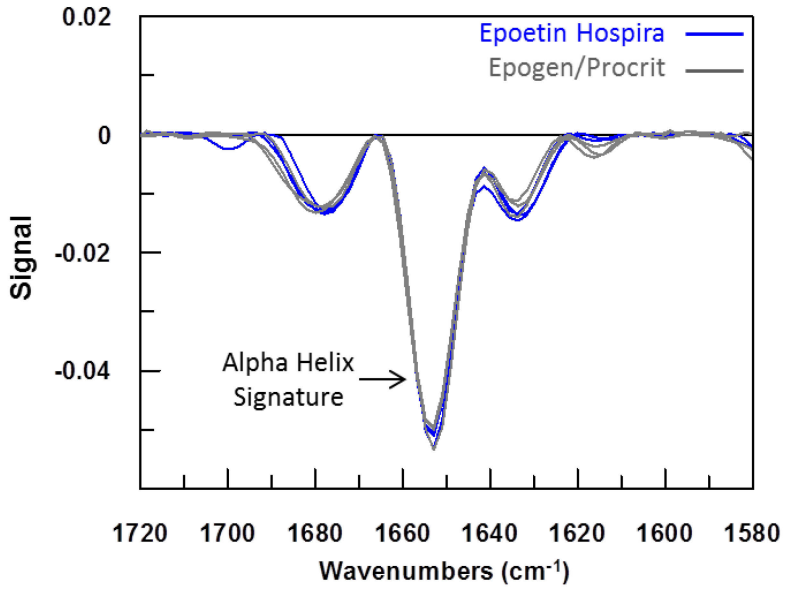
The average secondary structure content for the Epoetin Hospira lots is 62 ± 4% α-helix, 20 ± 2% total β-structure and 18 ± 2% random coil. This is comparable to the Epogen/Procrit reference product lots which average 61 ± 2% α-helix, 21 ± 1% total β-structure and 18 ± 1% random coil. These data demonstrate that the epoetin secondary structure is similar between Epoetin Hospira and the Epogen/Procrit reference product.

Figure 12. Far-UV CD Spectra for Representative Lots of Epoetin Hospira and the Epogen/Procrit Reference Product



The secondary structure of the epoetin protein in Epoetin Hospira and the Epogen/Procrit reference product was also compared using Fourier Transform Infrared (FTIR) spectroscopy, and representative results are shown in Figure 13. The key feature of these spectra is the wavenumber minimum at 1653 cm^{-1}, which is indicative a protein containing α-helix secondary structure. This feature is critical for epoetin, given that protein structure consists primarily of a four α-helix bundle. As indicated in Figure 13, both Epoetin Hospira and Epogen/Procrit exhibit this wavenumber minimum, providing further evidence that the secondary structure of the epoetin in Epoetin Hospira is similar to that of Epogen/Procrit.

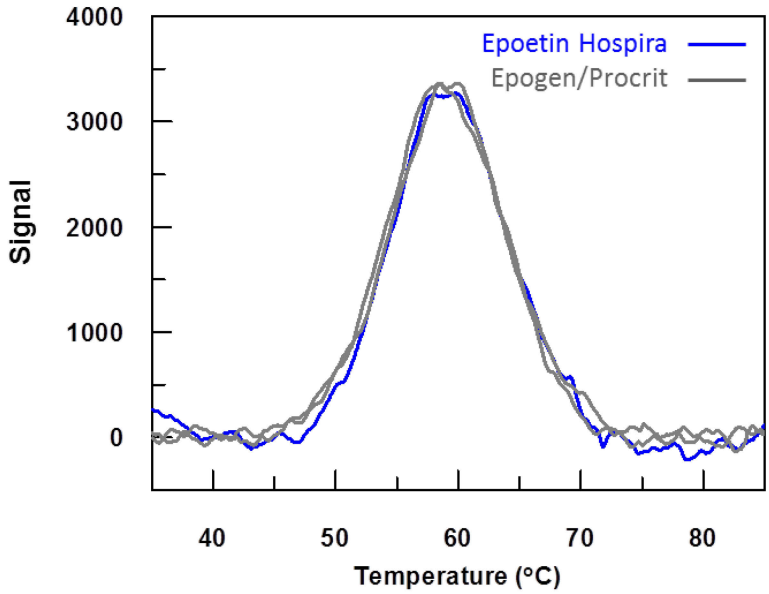
Figure 13. FTIR Spectra for Representative Lots of Epoetin Hospira and Epogen/Procrit Reference Product



The tertiary structure of the epoetin in Epoetin Hospira and the Epogen/Procrit reference product was evaluated using Differential Scanning Calorimetry (DSC). The DSC melting temperature (T_m) provides a measure of the conformational stability of the folded epoetin protein.

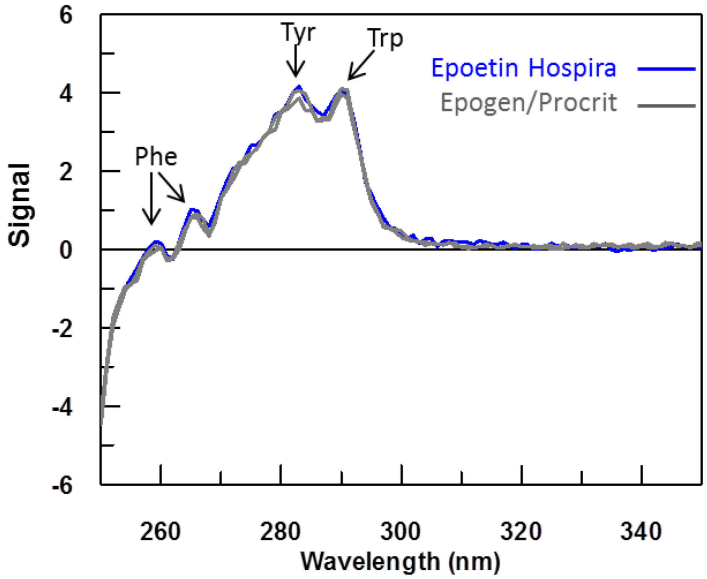
Representative DSC thermograms for Epoetin Hospira and the Epogen/Procrit reference product are shown in Figure 14. The range of observed T_m values for the Epoetin Hospira lots, 59.1 ± 0.3°C, is comparable to that observed for the Epogen/Procrit reference product lots, 59.2 ± 0.2°C. The results are consistent with published literature values that indicate an epoetin T_m of 60.6 ± 0.5°C at pH 7.2 (Lah et al., 2005). These data demonstrate that the epoetin tertiary structure and thermal stability are similar between Epoetin Hospira and the Epogen/Procrit reference product.

Figure 14. Representative Differential Scanning Calorimetry Thermograms for Epoetin Hospira and the Epogen/Procrit Reference Product



The tertiary structure of epoetin in Epoetin Hospira and the Epogen/Procrit reference product was also evaluated using Near-Ultraviolet Circular Dichroism (Near-UV CD). The spectral features in the near-UV region are mainly attributed to the structural environments surrounding tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. Representative Near-UV CD spectra for Epoetin Hospira and Epogen/Procrit reference product are provided in Figure 15 and show the same characteristic maxima corresponding to the Near-UV signals for the Trp, Tyr and Phe amino acid residues. The Near-UV results demonstrate that the epoetin protein present in Epoetin Hospira and the Epogen/Procrit reference product have similar tertiary structures.

Figure 15. Representative Near-UV Circular Dichroism Spectra for Epoetin Hospira and Epogen/Procrit Reference Product



Overall, the results from the higher order structural analyses demonstrate that the epoetin protein is highly similar between the two products.

4.3.3. Glycosylation

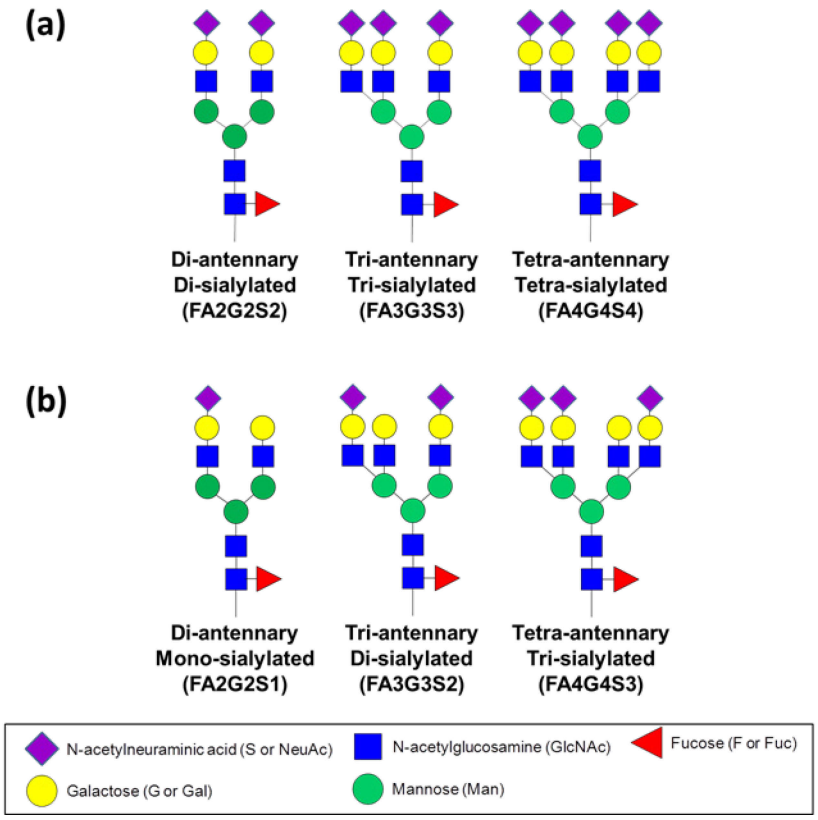
4.3.3.1. Overview of Epoetin Glycosylation

Glycosylation is the principal enzymatic post-translational modification that occurs during epoetin expression and was a central focus of the analytical similarity assessment. Epoetin is a highly glycosylated protein with three N-linked glycosylation sites and one O-linked glycosylation site, as described in Section 4.3.2.1. The N-linked glycan structures associated with the epoetin protein in Epoetin Hospira and the Epogen/Procrit reference product include a mixture of branched di-, tri- and tetra-antennary structures with terminal sialic acids on one or more branches. The terminal sialic acids, which cap the exposed galactose residues on the epoetin N-linked glycans, extend the *in vivo* half-life of circulating epoetin by preventing epoetin from binding to galactose receptors in the liver (Goldwasser et al., 1974; Mufson et al., 1987; Takeuchi et al., 1990; Fukuda et al., 1989). Epoetin with terminal sialic acids on the N-linked glycans removed (i.e., desialylated epoetin) is subject to rapid hepatic clearance and has decreased *in vivo* efficacy relative to fully sialylated epoetin (Fukuda et al., 1989). Consequently, the Total Sialic Acid Content was designated as a high-criticality attribute.

The maximum number of terminal sialic acids on each glycan is defined by the degree of glycan branching (e.g., tetra-antennary structures can accomodate a maximum of four terminal sialic acids). Partially sialylated structures with fewer than the maximum number of terminal sialic acids are also present at appreciable levels in both Epoetin Hospira and the Epogen/Procrit reference product. Several example di-, tri- and tetra-antennary epoetin

N-linked glycan structures that are fully and partially sialylated are shown in Figure 16(a) and Figure 16(b), respectively.

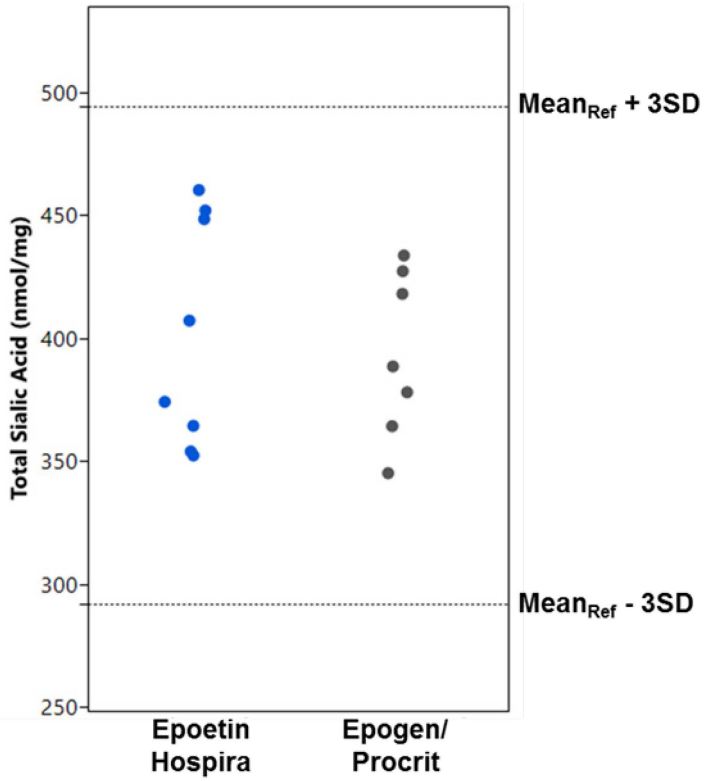
Figure 16. Example Fully and Partially Sialylated N-Linked Glycan Structures



4.3.3.2. Total Sialic Acid Content

A Total Sialic Acids analysis was performed by removal of the terminal sialic acids from epoetin followed by reversed phase high performance liquid chromatography (RP-HPLC) analysis of the isolated and labeled sialic acids. A summary of the Total Sialic Acid content results for the Epoetin Hospira and Epogen/Procrit reference product lots is shown in Figure 17. Levels of total sialic acids are similar for Epoetin Hospira and the Epogen/Procrit reference product. The Total Sialic Acid Content results for all Epoetin Hospira lots are within the mean $_{ref}$ ± 3SD quality range defined based on the reference product results.

Figure 17. Total Sialic Acid Content for Epoetin Hospira and the Epogen/Procrit Reference Product



The Total Sialic Acid Content results demonstrate that the epoetin protein present in Epoetin Hospira and the Epogen/Procrit reference product contains glycans with similar levels of terminal sialylation.

4.3.3.3. N-Glycolylneuraminic Acid

The predominant form of sialic acid found in human glycosylated proteins is N-acetylneuraminic acid (NeuAc). Recombinant glycosylated proteins expressed in non-human cell lines may contain low levels of the non-human form of sialic acid, N-glycolylneuraminic (NeuGc). NeuGc was designated as a high-criticality attribute since high levels of NeuGc in therapeutic proteins may be immunogenic. NeuGc is monitored as an impurity in Epoetin Hospira and levels of NeuGc were compared between Epoetin Hospira and the Epogen/Procrit reference product as part of the analytical similarity assessment. Levels of the NeuGc sialic acid species in Epoetin Hospira are lower than those observed in the Epogen/Procrit reference product.

4.3.3.4. Additional N-Linked Glycan Profile Comparisons

N-linked glycosylation attributes with lower criticality, including antennary structure and N-acetyllactosamine (Lac) repeats, were also evaluated as part of the analytical similarity assessment. N-linked glycan antennary structure is correlated with the maximum levels of

terminal sialic acids that can be attached to the glycans. However, the total sialic acid content of the epoetin glycans is not completely correlated with antennary structure due to the presence of partially sialylated structures in both Epoetin Hospira and the Epogen/Procrit reference products. Minor quantitative differences in N-linked glycan antennary structure were observed between Epoetin Hospira and Epogen/Procrit, but these did not impact the Total Sialic Acid Content.

Lac repeats are N-acetylglucosamine – galactose (GlcNAc-Gal) repeating units between the terminal galactose and sialic acid residues on one or more arms of the glycans. The presence of Lac repeats does not impact the number of terminal sialic acids on the glycans. Examination of epoetin samples with enriched Lac repeat structures using *in vitro* functional assays and *in vivo* mouse studies (described in BLA) demonstrated that Lac repeats do not impact receptor binding affinity or kinetics or the epoetin pharmacodynamic response *in vivo*.

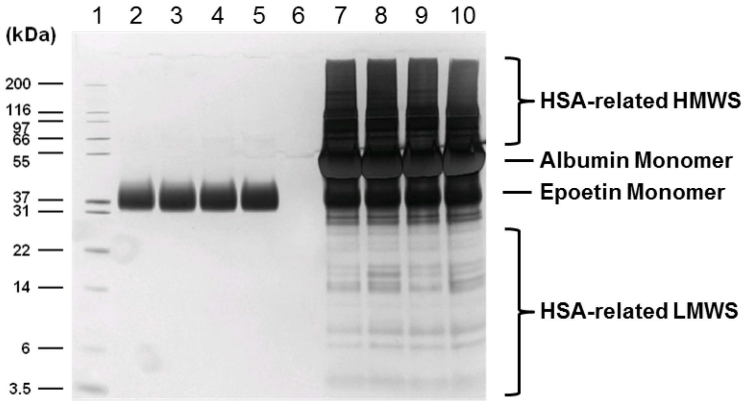
The demonstration of statistical equivalence between Epoetin Hospira and the Epogen/Procrit reference product for *In Vivo* Biopotency using the normocythemic mouse assay (described in Section 4.3.6.1), as well as the results from the supportive functional assay testing of enriched samples, provides strong evidence that any minor quantitative differences in the N-Linked Glycan Profile do not affect epoetin receptor binding affinity or kinetics, receptor activation or *in vivo* pharmacodynamics.

4.3.4. Product-Related Substances and Impurities

4.3.4.1. High Molecular Weight Species

Epoetin-related high molecular weight species (HMWS) may form when folded epoetin monomers associate to form multimeric species. These HMWS are monitored in the Epoetin Hospira DS and DP using Size Exclusion HPLC (SE-HPLC), SDS-PAGE and quantitative Western Blot methods. The results obtained from the orthogonal methods demonstrate that the levels of HMWS are low for all Epoetin Hospira lots (less than 1.3%). The SE-HPLC and SDS-PAGE methods cannot be used to measure HMWS in the Epogen/Procrit reference product due to the high levels of human serum albumin (HSA) present in the Epogen and Procrit formulations. A representative SDS-PAGE gel showing the HSA-related HMWS and other HSA-related low molecular weight impurities (LMWS) in the Epogen/Procrit reference product is provided in Figure 18 (lanes 7 – 10).

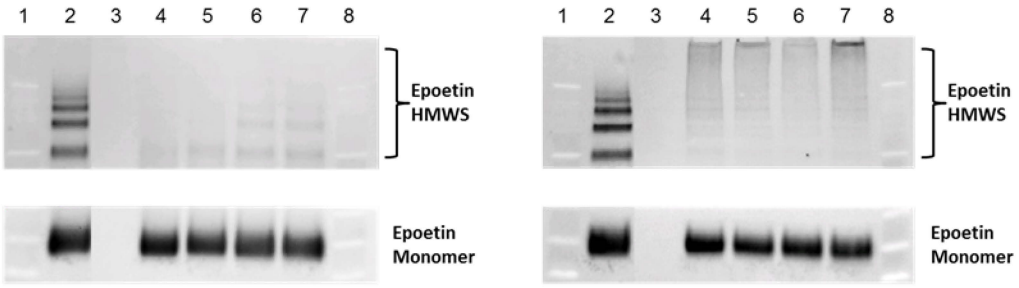
Figure 18. Representative SDS-PAGE Gel for Epoetin Hospira and Epogen/Procrit Reference Product Lots



Lane 1	Molecular weight marker
Lane 2	Epoetin Hospira 10,000 U/mL
Lane 3	Epoetin Hospira 10,000 U/mL
Lane 4	Epoetin Hospira 10,000 U/mL
Lane 5	Epoetin Hospira 40,000 U/mL
Lane 6	Epoetin Hospira formulation buffer
Lane 7	Epogen 10,000 U/mL
Lane 8	Epogen 10,000 U/mL
Lane 9	Epogen 10,000 U/mL
Lane 10	Procrit 40,000 U/mL

A quantitative Western Blot method was developed and qualified for the comparative assessment of HMWS in Epoetin Hospira and the Epogen/Procrit reference product. TheWestern Blot method provides selective and sensitive detection of epoetin-related species even in the presence of HSA (Figure 19). Levels of total HMWS in the Epoetin Hospira lots determined by Western blot analysis were demonstrated to be consistently below the limit of quantitation (LOQ) of the method (0.4%). HMWS levels for the Epogen/Procrit reference product ranged from < LOQ (0.4%) to 0.7%.

Figure 19. Representative Western Blot Analysis for Epoetin Hospira and Epogen/Procrit Reference Product Lots



Lane 1	Molecular weight marker
Lane 2	1% Standard
Lane 3	Epoetin Hospira formulation buffer
Lane 4	Epoetin Hospira 10,000 U/mL
Lane 5	Epoetin Hospira 10,000 U/mL
Lane 6	Epoetin Hospira 10,000 U/mL
Lane 7	Epoetin Hospira 10,000 U/mL
Lane 8	Molecular weight marker

Lane 1	Molecular weight marker
Lane 2	1% Standard
Lane 3	Epogen formulation buffer
Lane 4	Epogen 10,000 U/mL
Lane 5	Epogen 10,000 U/mL
Lane 6	Epogen 10,000 U/mL
Lane 7	Epogen 10,000 U/mL
Lane 8	Molecular weight marker

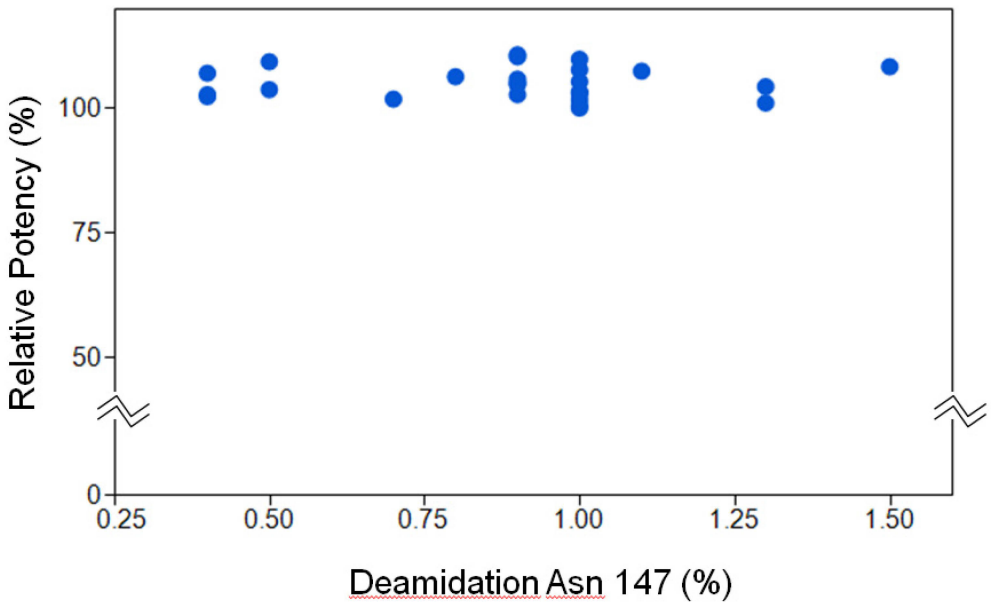
Overall, the Western Blot results demonstrate that the levels of epoetin-related HMWS in Epoetin Hospira are similar to or lower than those in the Epogen/Procrit reference product. Elimination of the HSA in the Epoetin Hospira formulation also eliminates the HSA-related HMWS and LMWS that are observed in the Epogen/Procrit formulations.

4.3.4.2. Asparagine Deamidation

Deamidation is a naturally occurring protein modification that results in the conversion of asparagine (Asn) residues to either aspartic acid (Asp) or isoaspartic acid (iso-Asp). Solvent-accessible Asn sites that are susceptible to deamidation *in vitro* generally deamidate readily *in vivo* following product administration (Liu et al., 2009). Asparagine deamidation was measured as part of the biosimilarity assessment. The mean levels of Asn 147 deamidation were 0.9% for the Epoetin Hospira lots and 0.4% for the Epogen/Procrit lots.

To assess the impact of the slightly higher levels of Asn 147 deamidation observed in Epoetin Hospira, samples with Asn 147 deamidation levels up to 1.5% were evaluated using the *in vitro* cell-based bioassay. These results, shown in Figure 20, demonstrate that there is no correlation between the amount of Asn 147 deamidation and epoetin biological activity at the low levels observed in the Epoetin Hospira product (1.5% or less). The minor difference in levels of Asn 147 deamidation observed between Epoetin Hospira and the Epogen/Procrit reference product therefore does not represent a biologically meaningful difference.

Figure 20. Comparison of *In Vitro* Cell-Based Biopotency and Percent of Deamidation at Asn 147 Results for Epoetin Hospira

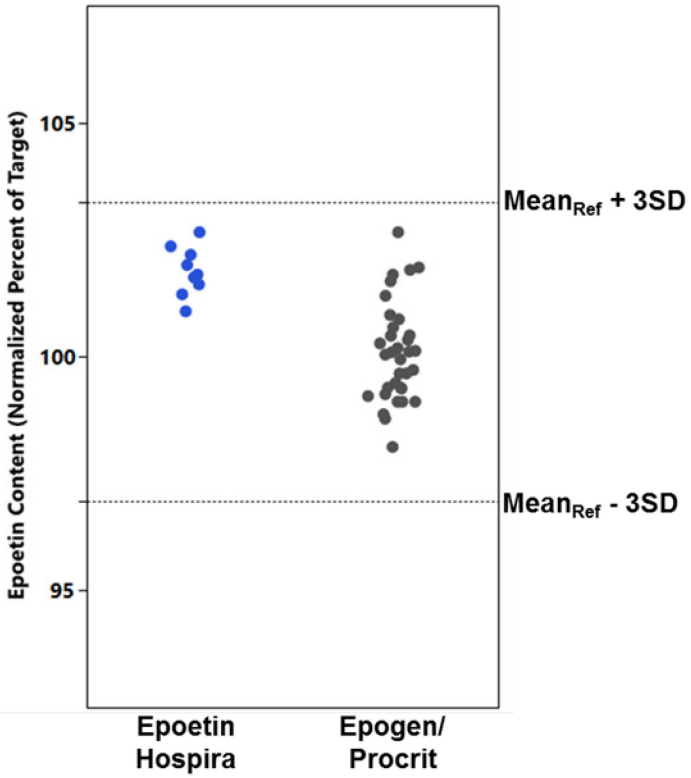


4.3.5. Drug Product Attributes

4.3.5.1. Epoetin Content

Epoetin content is expressed as the concentration of epoetin protein per unit volume of DP solution and is one measure of product strength. Epoetin content is determined using an reversed phase ultra performance liquid chromatography (RP-UPLC) method that was developed and validated to measure the epoetin content in Epoetin Hospira and the Epogen/Procrit reference product. The epoetin content target for Epoetin Hospira was revised during the BLA review in order to more closely match the reference product, as described in Section 3.2. A comparison of the epoetin content results for the Epoetin Hospira DP lots manufactured using the revised epoetin content target and the Epogen/Procrit reference product is shown in Figure 21. The epoetin content results for all of the Epoetin Hospira DP lots manufactured using the revised target are within the mean $_{ref}$ ± 3 SD quality range defined based on the reference product results. The results from the statistical analysis demonstrate that the epoetin content of the Epoetin Hospira DP is highly similar to the Epogen/Procrit reference product.

Figure 21. Epoetin Content Results for Epoetin Hospira and the Epogen/Procrit Reference Product



4.3.6. Functional Assays

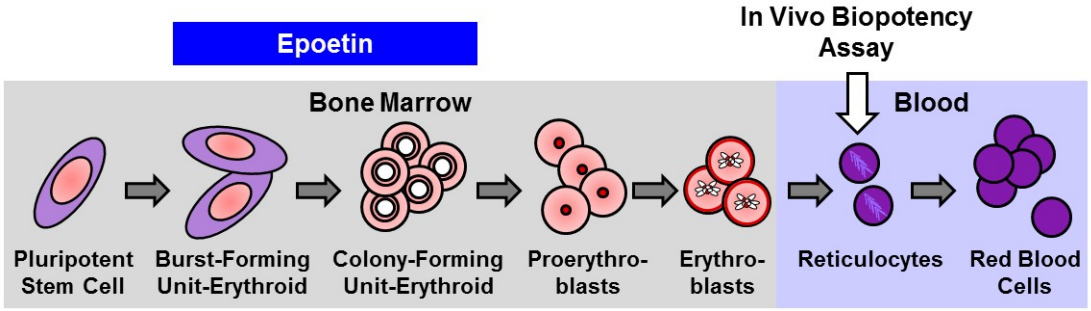
Multiple orthogonal functional assays were used to assess epoetin biological activity as part of the analytical similarity assessment. The assays used include an *in vivo* normocythemic mouse bioassay, an *in vitro* cell-based proliferation assay, a competitive epoetin receptor binding assay, and a Surface Plasmon Resonance (Biacore) assay for determination of epoetin receptor binding affinity (K_D) and kinetics (k_{on} and k_{off}). These functional assays are directly related to the epoetin therapeutic mechanism of action, which involves epoetin binding to high affinity receptors on the surface of colony-forming erythroid cells in the bone marrow followed by epoetin-dependent cell proliferation and erythropoiesis.

4.3.6.1. *In Vivo* Biopotency

The normocythemic mouse *in vivo* biopotency method is described in the Ph.Eur. Erythropoietin monograph and in the USP Erythropoietin Bioassays chapter <124>. The *in vivo* normocythemic mouse assay measures the ability of epoetin administered subcutaneously at titrated doses to increase reticulocyte count. The assay measures the combined effect of receptor binding, signaling in target cells, circulating half-life, and erythropoiesis and is a pharmacodynamic model for comparison of Epoetin Hospira and the Epogen/Procrit reference product *in vivo* biopotency.

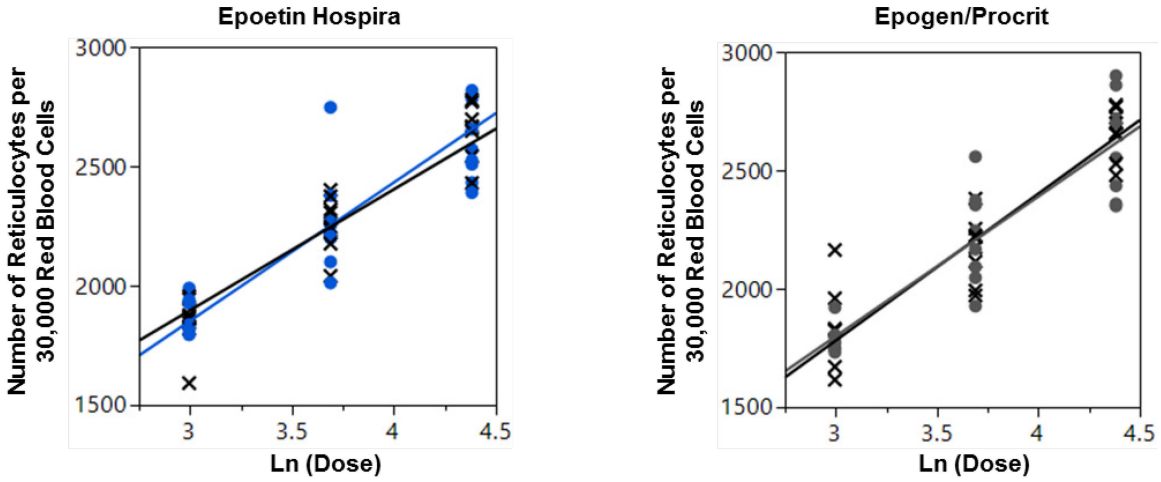
A schematic showing epoetin stimulation of the red blood cell maturation process, including reticulocyte formation, the endpoint measured in the normocythemic mouse assay, is shown in Figure 22.

Figure 22. Schematic Showing Epoetin Stimulation of the Red Blood Cell Maturation Process



In vivo biopotency results are reported as percent relative biopotency by direct comparison against the Epoetin Hospira biological reference standard dose response curve. Example dose response curves for Epoetin Hospira and the Epogen/Procrit reference product are shown in Figure 23.

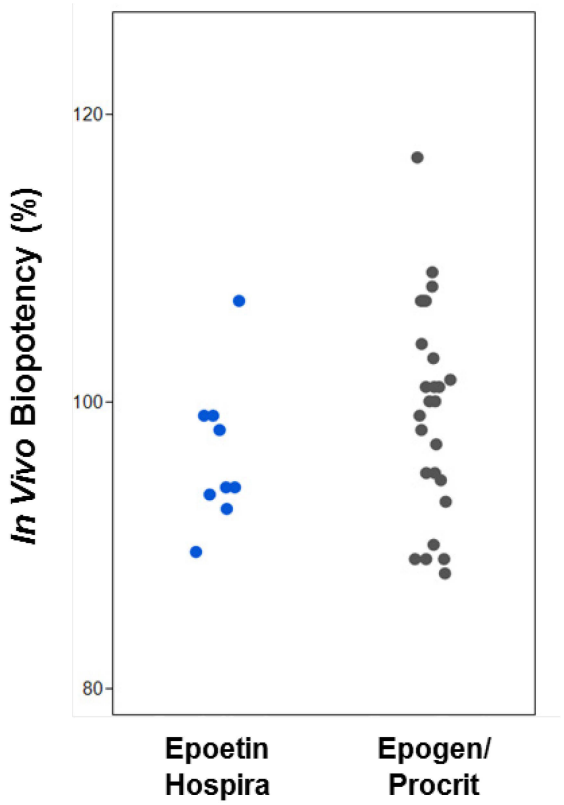
Figure 23. Dose Response Curves for Epoetin Hospira and Epogen/Procrit Lots



Epoetin Hospira – blue circle; Epogen/Procrit – grey circle; Hospira Biological Reference Standard – black ×

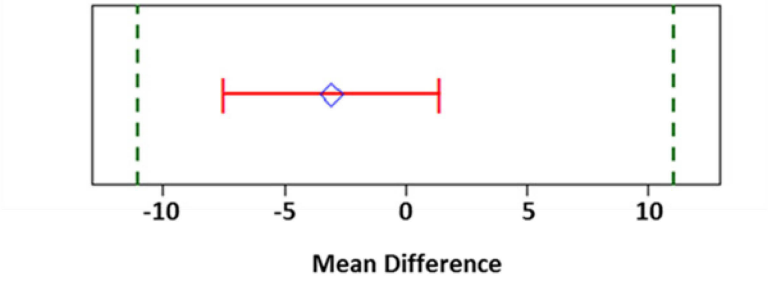
A graphical comparison of the *in vivo* biopotency results for the Epoetin Hospira and Epogen/Procrit reference product lots is shown in Figure 24. The Epoetin Hospira data set shown is limited to the lots manufactured with the revised epoetin content, as described in Section 3.2.

Figure 24. *In Vivo* Biopotency Results for Epoetin Hospira and the Epogen/Procrit Reference Product



In vivo biopotency was assigned as a Tier 1 attribute for statistical analysis because of the direct link between this attribute and the epoetin therapeutic mechanism of action and clinical performance. The *in vivo* biopotency results for the Epoetin Hospira and Epogen/Procrit reference product lots were evaluated via equivalence testing. An equivalence margin of ± 1.5 SD_{ref} defined by FDA was used, where SD_{ref} is the standard deviation of the reference product results. The equivalence testing results are provided in Figure 25 and show that the constructed 90% confidence interval (CI) around the mean difference between the Epoetin Hospira and Epogen/Procrit reference product lots results fall within the equivalence margins.

Figure 25. Summary of Equivalence Testing Results for *In Vivo* Biopotency



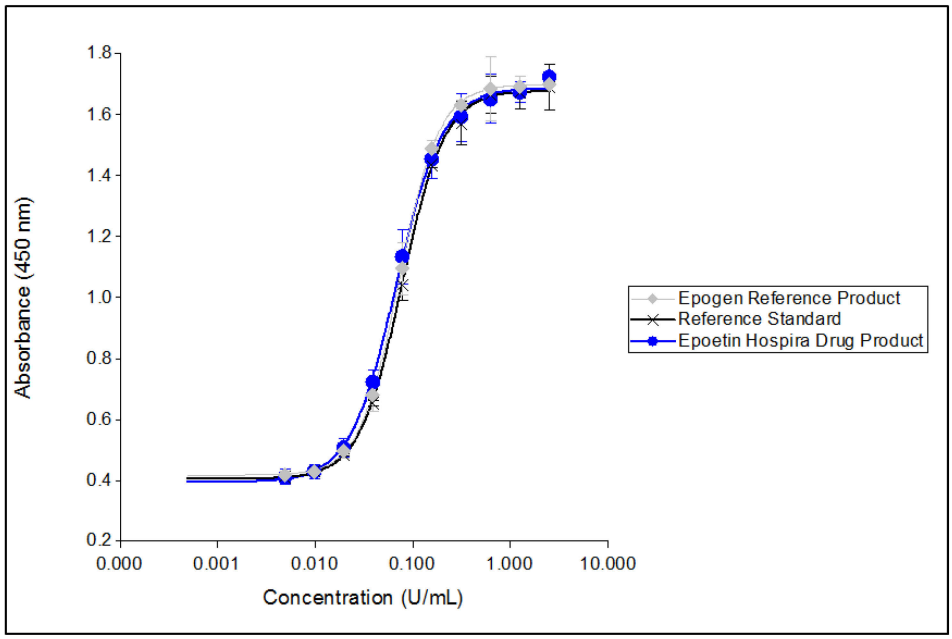
The results from the equivalence testing demonstrate that the *in vivo* biopotency of Epoetin Hospira is equivalent to that of the Epogen/Procrit reference product. *In vivo* biopotency is directly linked to the mechanism of action of the epoetin protein and demonstrates the combined effect of receptor binding, receptor activation, and persistence of drug at the receptor to facilitate erythropoiesis. This attribute is highly dependent on glycosylation and protein conformation for biological activity and the demonstration of equivalence confirms that other minor quantitative glycosylation and physicochemical differences between Epoetin Hospira and the Epogen/Procrit reference product (Table 11, Table 12, and Table 13) do not result in statistically significant differences in the *in vivo* biopotency or pharmacodynamics.

4.3.6.2. *In Vitro* Specific Activity

The UT-7 *in vitro* cell-based bioassay is the *in vitro* potency method described in the USP Erythropoietin Bioassays chapter <124>. The *in vitro* cell-based bioassay method measures epoetin-dependent proliferation of a UT-7 cell line resulting from epoetin receptor binding and signal transduction. The *in vitro* functional assays are not predictive of *in vivo* PK (clearance and half-life) and PD (efficacy) but do provide direct *in vitro* measurements of the effect of epoetin on the target receptor and/or cells.

The epoetin dose response is determined in the *in vitro* cell-based bioassay relative to the Epoetin Hospira biological reference standard. Representative dose response curves for the *in vitro* cell-based bioassay are shown in Figure 26 for Epoetin Hospira, the Epogen/Procrit reference product, and the biological reference standard.

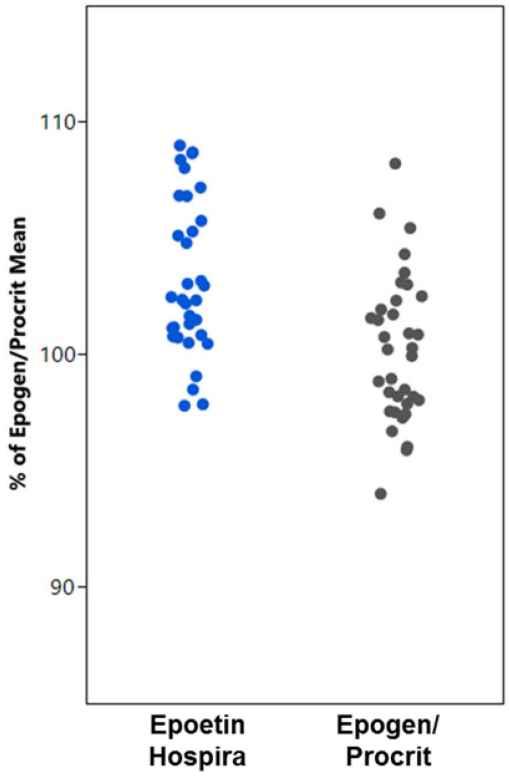
Figure 26. Representative *In Vitro* Cell-Based Bioassay Dose Response Curves for Epoetin Hospira, the Epogen/Procrit Reference Product and the Hospira Biological Reference Standard



In vitro specific activity (U/µg) is calculated by dividing the *in vitro* biopotency results (expressed in U/mL) by the measured epoetin content (µg/mL). *In vitro* specific activity is a measure of the intrinsic *in vitro* potency of the epoetin protein in the Epoetin Hospira and the Epogen/Procrit reference product formulations per unit mass. Comparison of specific activities provides an approach to closely examine the potential biological impact of small differences in higher order structure, low level product-related impurities or other physicochemical attributes between the epoetin protein from Epoetin Hospira and the Epogen/Procrit reference product.

A graphical comparison of the individual *in vitro* specific activity results for the Epoetin Hospira and the Epogen/Procrit reference product lots is shown in Figure 27.

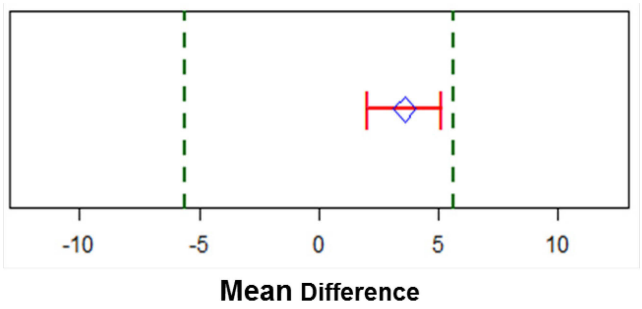
Figure 27. *In Vitro* Specific Activity Results for Epoetin Hospira and the Epogen/Procrit Reference Product



In vitro Specific Activity (U/µg) was also assigned as a Tier 1 attribute for statistical analysis because this attribute represents the intrinsic potency of the epoetin protein and has a direct link to the therapeutic mechanism of action. The *in vitro* Specific Activity results for Epoetin Hospira and the Epogen/Procrit reference product were further examined using equivalence testing. The equivalence margins were defined by FDA as ± 1.5 SD_{ref}, where SD_{ref} represents the standard deviation of the attribute estimated from lots of the Epogen/Procrit reference product, as described previously for *in vivo* biopotency. The equivalence testing results are provided in Figure 28 and show that the constructed 90% confidence interval (CI)

around the mean difference between the Epoetin Hospira and Epogen/Procrit reference product lots results fall within the equivalence margins.

Figure 28. Summary of Equivalence Testing Results for *In Vitro* Specific Activity



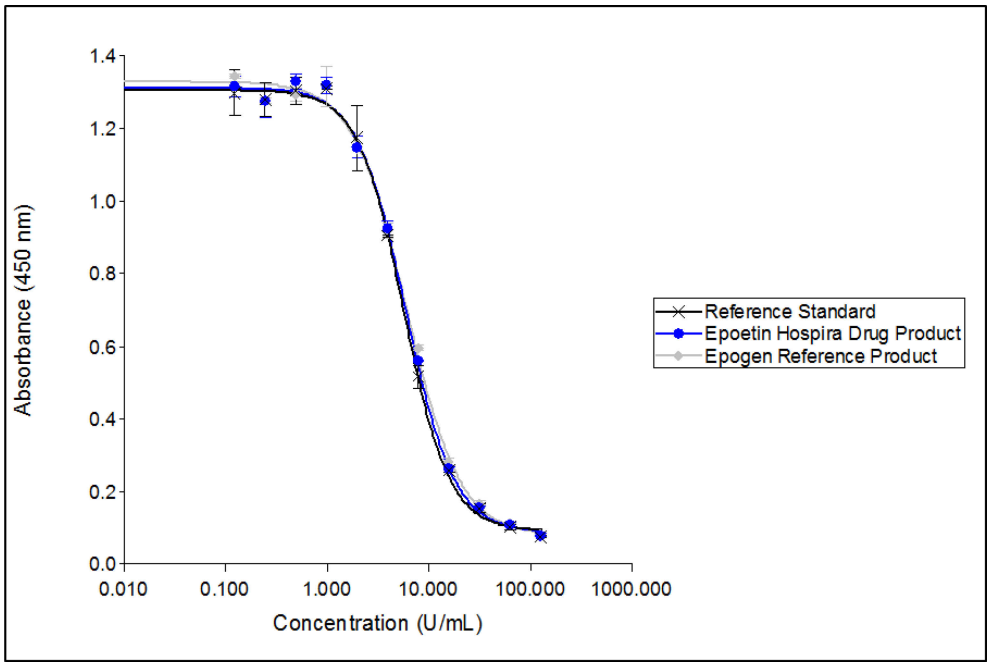
The results from the equivalence testing demonstrate that the *in vitro* specific activity of Epoetin Hospira is equivalent to that of the Epogen/Procrit reference product.

4.3.6.3. Receptor Binding

Orthogonal functional assay testing was performed using a competitive receptor binding ELISA method and Surface Plasmon Resonance (SPR) method to measure the binding affinity of epoetin for its receptor and the kinetics of epoetin binding to the receptor.

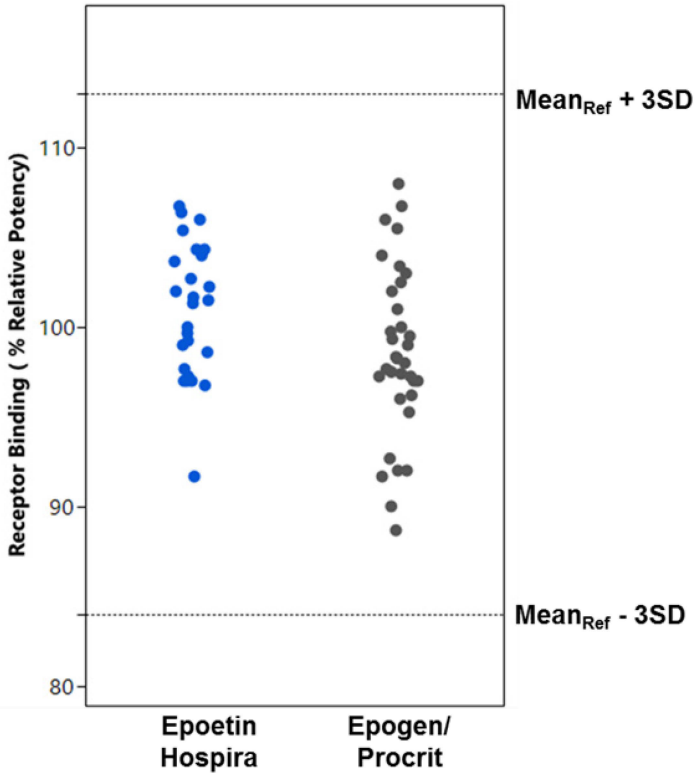
The receptor binding ELISA assay measures the competitive binding of the epoetin in Epoetin Hospira and the Epogen/Procrit reference product to an immobilized epoetin receptor relative to biotin-labeled epoetin. Relative potency is calculated by comparing the dose response curves for the Epoetin Hospira and Epogen/Procrit test samples to the Epoetin Hospira biological reference standard. Representative dose response curves for the receptor binding assay are shown in Figure 29.

Figure 29. Representative Receptor Binding Assay Dose Response Curves for Epoetin Hospira, the Epogen Reference Product and the Hospira Biological Reference Standard



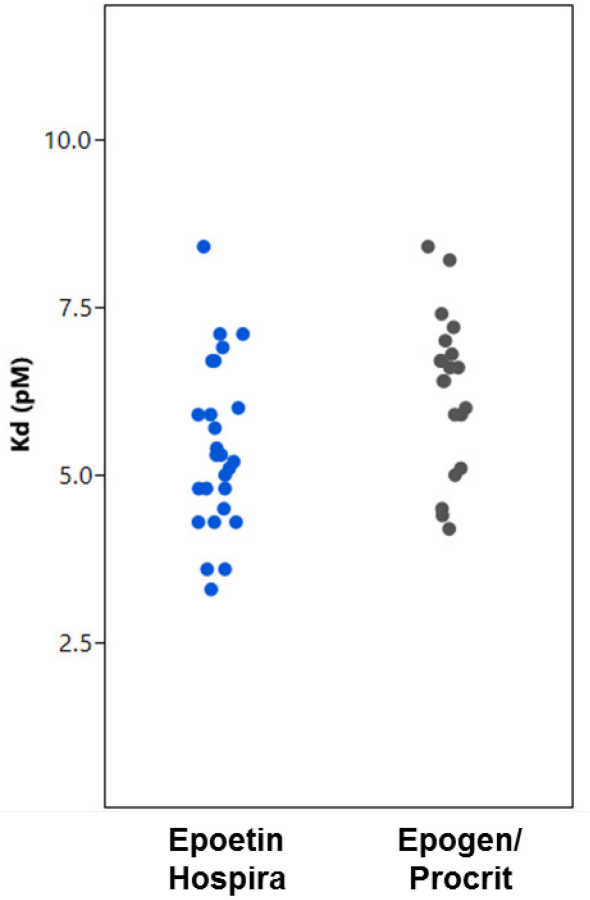
A graphical comparison of the individual Receptor Binding results for the Epoetin Hospira and Epogen/Procrit reference product lots are shown in Figure 30. The measured Receptor Binding for all the Epoetin Hospira lots are within the mean $_{ref}$ ± 3 SD quality range determined from the Epogen/Procrit reference product.

Figure 30. Receptor Binding Results for Epoetin Hospira and the Epogen/Procrit Reference Product



The kinetics of epoetin binding to the epoetin receptor was measured using a SPR method. The SPR results are analyzed using a binding model to determine the rate constant for receptor binding, k_{on} and the receptor off-rate for dissociation of the epoetin-receptor complex, k_{off}. The binding affinity constant, K_D is calculated by dividing k_{off} by k_{on}. A graphical comparison of receptor binding affinity results for the Epoetin Hospira and the Epogen/Procrit reference product lots is shown in Figure 31.

Figure 31. Binding Affinity (K_D) Results for Epoetin Hospira and the Epogen/Procrit Reference Product



The close agreement of the K_D values for Epoetin Hospira and the Epogen/Reference reference product demonstrates that the epoetin:receptor affinities are similar for both products. The comparative SPR results provide additional evidence that the structure and function of the epoetin protein present in Epoetin Hospira is highly similar to that of the Epogen/Procrit reference product.

4.4. Analytical Assessment Conclusion

A comprehensive analytical similarity assessment was conducted as part of the overall Epoetin Hospira development program. This assessment included comparative analyses of the primary structure, higher order structure, post-translational modifications, product-related substances and impurities, drug product attributes, and functional activity of Epoetin Hospira and the Epogen/Procrit reference product.

The results of the analytical similarity assessment confirm that the amino acid sequence, sites of glycosylation, disulfide bonds and higher order structure of the epoetin protein in Epoetin Hospira are highly similar to those of the epoetin in the Epogen/Procrit reference product. In addition, the highly critical sialic acid content of the N-linked glycans on the epoetin present

in Epoetin Hospira is highly similar to that of the Epogen/Procrit reference product. The attributes of the Epoetin Hospira drug product that are linked to strength and safety (epoetin content and HMWS) were also demonstrated to be highly similar to the reference product. Finally, the comprehensive functional testing that included multiple orthogonal and highly sensitive *in vivo* and *in vitro* assays demonstrated that the biopotency and receptor binding affinity and binding kinetics of the epoetin in Epoetin Hospira are highly similar to those of the Epogen/Procrit reference product. Equivalence testing was performed on the two designated Tier 1 functional attributes, *in vivo* biopotency and *in vitro* specific activity, considered most relevant to the epoetin MOA and clinical activity. Both of these key Epoetin Hospira attributes were demonstrated to be equivalent to the Epogen/Procrit reference product.

A minor number of quantitative physicochemical differences were observed between Epoetin Hospira and the reference product. These differences did not impact higher order structure, functional activity, receptor binding, or stability as measured by multiple comparative *in vitro* and *in vivo* bioassays and physicochemical methods.

Overall, the results of the analytical similarity assessment demonstrate that Epoetin Hospira is highly similar to the US-licensed Epogen/Procrit reference product.

5. BIOSIMILARITY BETWEEN EPOETIN HOSPIRA AND EPOGEN REFERENCE PRODUCT BASED ON RESULTS OF NONCLINICAL ASSESSMENTS

Two GLP-compliant 13-week repeat-dose comparative toxicity studies (one in Sprague Dawley CD rats [*Study ITR: 70882*] and one in beagle dogs [*Study ITR: 60486*]) were conducted, which were in alignment with the *FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (FDA 2015a) (Table 14).

Pharmacodynamic (PD), pharmacokinetic (PK)/toxicokinetic (TK), immunogenicity, and toxicity assessments were included in the two studies. The nonclinical studies described in the FDA Summary Basis of Approval for the Epogen (1989) original submission informed the species, dose levels, and dose regimen studied in the Epoetin Hospira biosimilar program.

Table 14. Design of 13-Week Repeat Dose Nonclinical Studies

Species/Strain	Method of Administration	Duration of Dosing	Doses (IU/kg)*	Endpoints
Sprague-Dawley CD Rats	Subcutaneous	3 times weekly over a 13-week dosing period with 4-week recovery period	Epoetin Hospira: 0, 150, 450 and 1500/900 Epogen: 0, 150, 450 and 1500/900	Toxicity PK PD Immunogenicity
Beagle Dogs	Intravenous	3 times weekly over a 13-week dosing period with 4-week recovery period	Epoetin Hospira: 0, 150, 450 and 1500/900 Epogen: 0, 150, 450 and 1500/900	

* High dose, dose level was reduced from 1500 IU/kg to 900 IU/kg due to severe adverse clinical observations due to the known pharmacologic action of the drug products.

Toxicity profiles for Epoetin Hospira and Epogen were similar based on the results of standard assessments (e.g., mortality, clinical observations, body weight, food consumption, clinical pathology, and anatomic pathology) at three dose levels (150, 450, and 1500/900 IU/ kg, three times weekly) in rat (SC administration, Table 15) and dog (IV administration, Table 16).

Table 15. 13-Week Rat SC Study: Comparative Toxicology Observations

Dose	Epoetin Hospira	Epogen
All dose levels	▪ Red discoloration of the skin of the limbs, pinnae, tail and/or whole body, reduced grooming activities, hair loss and fur discoloration (beige/red) of limbs, head and/or tail.	▪ Red discoloration of the skin of the limbs, pinnae, tail and whole body, pallor of the limbs and/or whole body, slight hypoactivity, emaciation, dehydration, hypothermia, hunched posture, reduced grooming activities and reduction in fecal output.
Doses of 450 and 1500/900 IU/kg	▪ Slight hypoactivity, emaciation, dehydration, swelling of the hind limbs, loss of limb function, hypothermia and/or a reduction in fecal output. ▪ Incidence and severity of these findings were higher in the 1500/900 IU/kg treated ▪ Majority of the clinical signs noted no longer seen during the 4-week recovery period	▪ Swelling of the limbs, loss of limb function and/or salivation. ▪ Incidence and severity of these findings were higher in the 1500/900 IU/kg treated ▪ Majority of the clinical signs noted during 4-week recovery period, except for pallor of the whole body

Study ITR-70882

Table 16. 13-Week Dog IV Study: Comparative Toxicology Observations

Dose	Epoetin Hospira	Epogen
Dose of 150 IU/kg	▪ No clinical signs attributed to Epoetin Hospira	▪ No clinical signs attributed to Epogen
Doses of 450 and 1500/1900 IU/kg	▪ Slight to moderate increase in the incidence, frequency and severity of emaciation, diarrhea	▪ Slight to moderate increase in the incidence, frequency and severity of emaciation, diarrhea (revealed by the presence of loose and/or liquid feces in the cage-tray)

Table 16. 13-Week Dog IV Study: Comparative Toxicology Observations

Dose	Epoetin Hospira	Epogen
Doses of 1500/900 IU/kg	▪ Similar profile in clinical signs as Epogen at this dose level: hypoactivity, loss of limb function (1 male), pale discoloration of the feces, reduced fecal output, dehydration, red gums and/or discoloration (yellow) of the teeth. ▪ No clinical signs during the 4-week recovery period	▪ Similar profile in clinical signs as Epoetin Hospira at this dose level: hypoactivity, loss of limb function (1 male), discoloration of the feces, reduced fecal output, dehydration, red gums and/or discoloration (yellow) of the teeth ▪ 3 of 4 recovery remained thin throughout 4-week recovery period.

Study ITR-60486

In *Study ITR-70882* in rats, SC administration of Epoetin Hospira resulted in considerable increases in four PD measures (red blood cell and reticulocyte counts, Hb, and Hct) compared to the effect of Epogen at Week 13 in all dose groups. The lower values observed in PD for Epogen compared to Epoetin Hospira in the rat study are likely due to the increased incidence of immunogenicity observed following SC administration of Epogen. IV administration in beagle dogs in *Study ITR: 60486* demonstrated similarity between Epoetin Hospira and Epogen with respect to the same four *in vivo* PD parameters.

In *Study ITR-70882* in rats, administration of Epoetin Hospira on Day 1 resulted in lower C_{max} and AUC_{0-t} values when compared to Epogen. However, the values of C_{max} and AUC_{0-t} in the Epogen-treated animals were lower on Day 26 and much lower on Day 89 when compared to the effect of Epoetin Hospira. On Day 1, the lower C_{max} and AUC_{0-t} values observed with Epoetin Hospira were likely due to the lower protein content in the Epoetin Hospira test material compared to Epogen. The lower C_{max} and AUC_{0-t} values associated with Epogen on Days 26 and 89 may be due to the increased incidence of immunogenicity observed following SC administration of Epogen in rats. Much higher levels of anti-drug antibodies (ADAs) as well as an increase in neutralizing antibody (NAb) were observed for Epogen, which has a different formulation containing human serum albumin (HSA) as compared to Epoetin Hospira.

In *Study ITR-60486* in dogs, IV administration of Epoetin Hospira three times per week for 13 weeks showed that the values of mean C_{max} and AUC_{0-t} were slightly lower on Days 1, 26, and 89 compared to Epogen. The slightly lower values of C_{max} and AUC_{0-t} observed with Epoetin Hospira dogs may be the result of the lower protein content in the Epoetin Hospira test material administered to the dogs. The protein content was adjusted for the subsequent clinical and commercial material. There was minimal effect of immunogenicity on their PD and PK/TK parameters for both Epogen and Epoetin Hospira in both genders in the dog study. The immunogenicity profiles following IV administration of Epoetin Hospira and Epogen were considered similar. The lack of an immune response following Epogen dosing in dogs was likely due to the IV administration of Epogen even though it contains HSA in its formulation.

The overall findings from the two comparative GLP-compliant toxicity studies with the incorporation of PD, PK/TK, and immunogenicity assessments demonstrate that Epoetin Hospira and Epogen produced similar effects in rats and dogs. Of note, a species difference was observed in the rat with regard to PK/TK and PD under subcutaneous conditions where human serum albumin (HSA) is an excipient uniquely in the reference product. The use of HSA in the reference product likely contributed to increased immunogenicity for Epogen reference product in the rat, thereby influencing the comparative PK, TK and PD in the rat. This was not seen in dogs under intravenous conditions where the PK/TK and PD were similar between the two treatment arms. The clinical data better inform the assessment of PK/PD similarity. Results from these studies support the safety of Epoetin Hospira for the intended clinical use. The comparative nonclinical data provides additional support for the demonstration of biosimilarity between Epoetin Hospira and the Epogen reference product.

6. BIOSIMILARITY BETWEEN EPOETIN HOSPIRA AND EPOGEN REFERENCE PRODUCT BASED ON RESULTS OF CLINICAL STUDIES

As discussed in Section 2.4.3 of this Briefing Document, the clinical development program for Epoetin Hospira comprised 7 clinical studies, among them 2 PK/PD studies in healthy subjects and 4 clinical efficacy and safety studies of subjects with CKD on HD (Table 17). One pilot PK study was conducted in subjects with CKD on HD. The pilot PK study informed a change in formulation to target epoetin protein content.

Table 17. Clinical Studies in the Epoetin Hospira Clinical Development Program

Study*	Description	Route of Administration	Type/Number of Subjects
EPOE-12-02	Comparative single-dose PK/PD study	Subcutaneous	HS, 81 randomized
EPOE-14-01	Comparative multiple-dose PD/PK study	Subcutaneous	HS, 129 randomized
EPOE-10-13	Comparative safety and efficacy study	Subcutaneous	CKD on HD; 320 randomized
EPOE-10-01	Comparative safety and efficacy study	Intravenous	CKD on HD; 612 randomized
EPOE-11-04	Supportive long-term safety study	Subcutaneous	CKD on HD; 173 enrolled
EPOE-11-03	Supportive long-term safety study	Intravenous	CKD on HD; 414 enrolled
EPOE-10-08*	Pilot comparative PK study	Intravenous	CKD on HD; 105 randomized

CKD, chronic kidney disease; HD, hemodialysis; HS, healthy subjects.
*Note: All studies, except EPOE-10-08, used the same late stage development formulation. EPOE-10-08 was a pilot PK study using an early formulation.

The clinical program was designed to establish biosimilarity between Epoetin Hospira and Epogen/Procrit reference product. Equivalence study designs were utilized to robustly evaluate PK/PD similarity as well as comparative clinical efficacy.

6.1. Clinical Pharmacology

PK/PD analyses were conducted in a single-dose, 2-period crossover study of 81 healthy male subjects (Study EPOE-12-02) and a multiple-dose, parallel group PK/PD study in 129 healthy male subjects (Study EPOE-14-01) (refer to Section 6.1.2 for a description of the studies). Single and multiple dose PK/PD assessments in healthy subjects are the most discerning for characterizing the PK and PD response to epoetin and identifying any potential product differences, should they exist.

Healthy subjects lack the comorbidities and the concomitant medications that can confound PK results and also maintain the functional bone marrow that might otherwise confound PD results. In addition, the PK and PD are well established for Epogen/Procrit in multiple

patient populations. Demonstration of PK and PD equivalence of Epoetin Hospira and Epogen in healthy subjects supports that equivalent epoetin concentration profiles and PD response profiles for the 2 products can be expected in all populations and conditions of use.

Hemoglobin and reticulocyte count are well-established PD markers reflective of the known mechanism of action of epoetin on erythropoietic response and are correlated with clinical response, further emphasizing the highly discerning nature of these studies to detect even small differences between products (Cheung et al, 1998; Cheung et al, 2001; Ramakrishnan et al., 2004; Sorgel et al., 2009). Hemoglobin was selected as an appropriate primary PD parameter to assess epoetin-stimulated erythropoiesis in the multiple-dose PK/PD study EPOE-14-01. Reticulocyte count expressed as percent of erythrocytes (Ret%) was the primary PD marker in the single-dose PK/PD study EPOE-12-02.

6.1.1. Summary of Clinical Pharmacology

PK/PD similarity between Epoetin Hospira and Epogen reference product was concluded under single (Study EPOE-12-02) and multiple (EPOE-14-01) fixed-dose conditions in healthy subjects.

- PK similarity was demonstrated with assessment of area under the serum concentration-time curve (AUC) and the observed maximum epoetin concentration (C_{max}).

- PD similarity was demonstrated with assessment of reticulocyte count by area under the effect-time curve (AUEC) and observed maximum effect (E_{max}) following single-dose administration, and area under the effect curve for hemoglobin ($AUEC_{Hb}$) over four weeks of dosing.

Sensitivity analyses demonstrated no impact of immunogenicity on PK or PD endpoints.

6.1.2. Overview of PK/PD Studies

PK/PD similarity between Epoetin Hospira and Epogen reference product was assessed under single dose (Study EPOE-12-02) and multiple fixed-dose (EPOE-14-01) conditions in healthy subjects (Table 18).

Single Dose PK/PD Study (EPOE-12-02): was designed as a single-center, open-label, randomized, 2-period, 2-sequence crossover study. Eighty-one healthy male subjects were randomized to receive a single 100 U/kg dose of either Epoetin Hospira or Epogen, administered by SC injection, on Day 1 of Period 1 or Period 2 according to the subject's randomized sequence.

The primary endpoints were the PK parameters of:

- Area under the concentration-time curve from time zero to time of last measurable concentration (AUC_{0-t}), and
- C_{max} determined from baseline-adjusted epoetin concentration (BAEC).

The secondary endpoints were the PD parameters of:

- $AUEC_{0-t}$ determined from reticulocyte count as percent of erythrocytes **(**Ret%), and
- E_{max} for Ret%.

Multiple Dose PK/PD (EPOE-14-01): was designed as a single-center, open-label, randomized, parallel group study. A total of 129 healthy, male subjects were randomized to receive either Epoetin Hospira or Epogen 100 U/kg SC 3 times weekly (TIW) for 4 weeks.

The primary endpoint was the PD parameter of:

- Area under the effect curve for hemoglobin ($AUEC_{Hb}$).

The secondary endpoints were the PK parameters of:

- Area under the concentration-time curve from time zero to 48 hours (AUC_{0-48}), and
- C_{max}.

Table 18. Summary of PK/PD Studies in Epoetin Hospira Clinical Development Program

	Single Dose PK/PD (EPOE-12-02) (N = 81)	Multiple Dose PK/PD (EPOE-14-01) (N = 129)
Subjects	Healthy male	Healthy male
Study design	Single-dose, 2-period crossover PK/PD study that evaluated the PK and PD equivalence of Epoetin Hospira and Epogen in healthy male subjects; average equivalence statistical approach to compare PK/PD parameters	Definitive multiple-dose, parallel group PK/PD study that evaluated the PK/PD equivalence of Epoetin Hospira and Epogen in healthy male subjects; average equivalence statistical approach to compare PK/PD parameters
PK and PD Populations	The PK (PD) population on which the PK (PD) analysis was conducted consisted of subjects who received both treatments and had sufficient data to calculate the primary PK (PD) parameters and excluded subjects with a positive ADA result for any antibody sample.	The PK (PD) population on which the PK (PD) analysis was conducted consisted of subjects who had sufficient data to calculate the primary PK (PD) parameter(s) and excluded subjects who had a positive ADA.
Dosing	Single SC dose of 100 U/kg epoetin	12 fixed doses of 100 U/kg administered TIW SC over 26 days
PK Endpoints	AUC_{0-t}, C_{max} for epoetin	AUC_{0-48}, C_{max} for epoetin
Timing of PK Assessments	Serial blood samples collected before and after dosing during each crossover study period	Serial blood samples obtained prior to each dose and after dosing on Day 26
PD Endpoints	$AUEC_{0-t}$, E_{max} reticulocyte count	$AUEC_{Hb}$ hemoglobin
Timing of PD Assessments	Serial blood samples obtained before and after dose administration through Day 20 of each study period	Serial blood samples obtained prior to each dose and 48 hours after dose administration through Day 26 of each study period
Other Assessments	Immunogenicity, safety	Immunogenicity, safety

AUC_{0-t}, area under the concentration-time curve from time zero to time of last measurable concentration; AUC_{0-48}, area under the concentration-time curve from time zero to 48 hours; AUEC, area under the effect-time curve; C_{max}, maximum observed concentration; E_{max}, maximum observed effect; TIW, three times per week

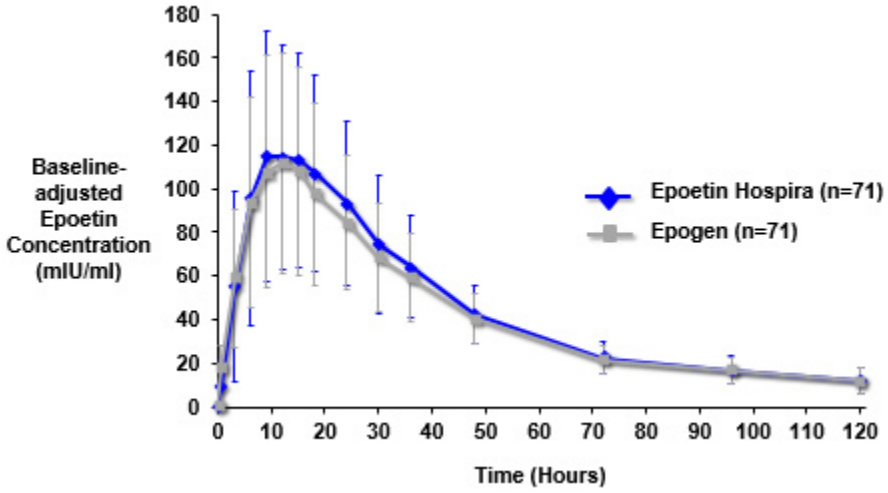
6.1.3. Single-Dose PK/PD Results (EPOE-12-02)

Single-Dose Pharmacokinetics

Original Analysis

Mean BAEC profiles over time were similar between the Epoetin Hospira and Epogen treatment groups (Figure 32).

Figure 32. Single Dose PK: Mean (± SD) Baseline-Adjusted Epoetin Concentration Profiles after Single Subcutaneous Administration of 100 U/kg of Epoetin Hospira or Epogen to Healthy Male Subjects in the Single Dose PK/PD Study (Pharmacokinetic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing was at Time 0.
Study EPOE-12-02.

The PK population on which the PK analysis was conducted consisted of subjects who received both treatments and had sufficient data to calculate the primary PK parameters (C_{max} and AUC_{0-t}) and excluded subjects with a positive anti-drug antibody (ADA) result for any antibody sample. Pharmacokinetic similarity between Epoetin Hospira and Epogen following single-dose administration was demonstrated based on 90% CIs for the geometric mean ratios (GMRs) (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both AUC_{0-t} and C_{max} derived from BAEC in serum (Table 19), and was supported by sensitivity analysis conducted in subjects who received at least one dose of study drug (data included in the BLA).

Table 19. Primary Pharmacokinetic Evaluation in the Single Dose PK/PD Study (Original Analysis, Pharmacokinetic Population)

Parameter	Statistic	Epoetin Hospira (N = 71)	Epogen (N = 71)	Ratio	90% CI for Ratio
AUC_{0-t} (mIU*hr/mL)	Geometric Mean	4998.51	4754.33	1.05	(1.01, 1.11)
C_{max} (mIU/mL)	Geometric Mean	120.52	110.86	1.09	(1.01, 1.18)

Study EPOE-12-02

Supplemental Analysis

A supplemental immunogenicity assessment was performed using updated validated stringent in-study cutpoints to identify ADA-positive subjects. Based on these supplemental immunogenicity results, a supplemental analysis for the PK primary endpoints was

conducted to exclude ADA-positive subjects from the PK population. The results of the supplemental analysis for the PK primary endpoints are shown in Table 20.

The 90% confidence intervals for both AUC_{0-t} and C_{max} for the supplemental analysis were within the acceptance limits of 0.80 - 1.25, confirming PK equivalence of Epoetin Hospira and Epogen.

Table 20. Primary Pharmacokinetic Evaluation in the Single Dose PK/PD Study (Supplemental Analysis, Pharmacokinetic Population)

Parameter	Statistic	Epoetin Hospira (N = 61)	Epogen (N = 61)	Ratio	90% CI for Ratio
AUC_{0-t} (mIU*hr/mL)	Geometric Mean	4974.35	4709.66	1.06	(1.01, 1.12)
C_{max} (mIU/mL)	Geometric Mean	120.77	108.23	1.12	(1.03, 1.23)

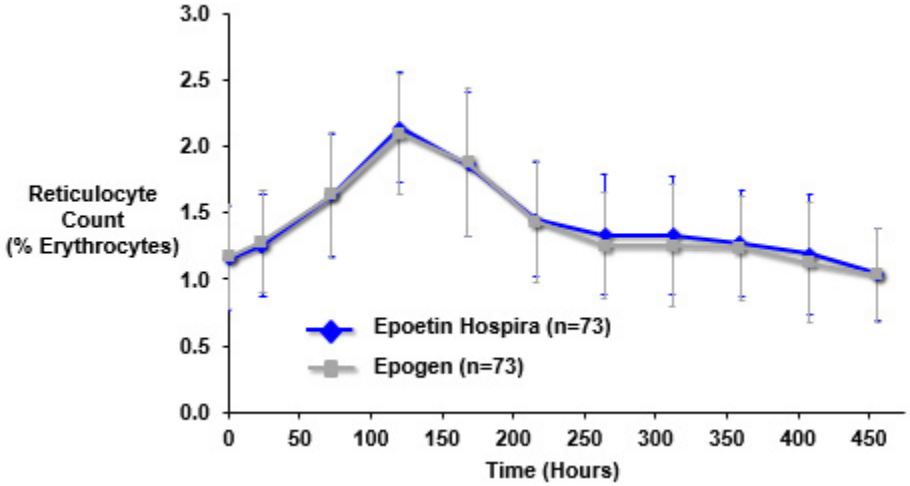
Study EPOE-12-02

Single-Dose Pharmacodynamics (EPOE-12-02)

Original Analysis

The PD population on which the PD analysis was conducted consisted of subjects who received both treatments and had sufficient data to calculate the primary PD parameters (E_{max} and $AUEC_{0-t}$ for Ret%) and excluded subjects with a positive ADA result for any antibody sample. Time profiles of Ret% were similar between the Epoetin Hospira and Epogen treatment groups over a 20-day period following study drug administration (Figure 33). Pharmacodynamic results of Study EPOE-12-02 are consistent with the findings of previously published work, reporting an increase in reticulocyte count within 3 to 4 days with a return to baseline by approximately 22 days (528 hours) following SC administration of recombinant human erythropoietin to healthy subjects (Cheung et al., 1998; Ramakrishnan et al., 2004).

Figure 33. Single Dose Pharmacodynamics: Mean (± SD) Ret% after Single Subcutaneous Administration of 100 U/kg of Epoetin Hospira or Epogen in the Single Dose PK/PD Study (Pharmacodynamic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing at Time 0.
Study EPOE-12-02.

PD similarity between Epoetin Hospira and Epogen following single-dose administration was demonstrated based on FDA-requested 90% CIs for GMRs (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both $AUEC_{0-t}$ and E_{max} derived from Ret% (Table 21) and was supported by sensitivity analysis conducted in subjects who received at least one dose of study drug (data included in the BLA). Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated based on pre-specified 95% CIs for GMRs (Table 21).

Table 21. Pharmacodynamic Evaluation in the Single Dose PK/PD Study (Original Analysis, Pharmacodynamic Population)

Parameter	Geometric Mean		Ratio	90% CI for Ratio*	95% CI for Ratio**
	Epoetin Hospira (N = 73)	Epogen (N = 73)			
$AUEC_{0-t(Ret)}$ (%*hr)	644.25	635.28	1.01	(0.98, 1.05)	(0.98, 1.05)
$E_{max\ (Ret)}$ (%)	2.18	2.13	1.02	(0.99, 1.05)	(0.98, 1.06)

*90% CI requested by FDA during BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed during the development program.
Study EPOE-12-02

Supplemental Analysis

A supplemental immunogenicity assessment was performed using updated validated stringent in-study cutpoints to identify ADA-positive subjects. Based on these supplemental immunogenicity results, a supplemental analysis for the PD primary endpoints was

conducted to exclude ADA-positive subjects from the PD population. The results of the supplemental analysis for the PD primary endpoints are shown in Table 22.

The FDA-requested 90% CIs for both $AUEC_{0-t}$ and E_{max} for the supplemental analysis were within the acceptance limits of 0.80 - 1.25, supporting PD equivalence of Epoetin Hospira and Epogen. Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated based on the pre-specified 95% CIs for GMRs (Table 22).

Table 22. Pharmacodynamic Evaluation in the Single Dose PK/PD Study (Supplemental Analysis, Pharmacodynamic Population)

Parameter	Geometric Mean		Ratio	90% CI for Ratio*	95% CI for Ratio**
	Epoetin Hospira (N = 62)	Epogen (N = 62)			
$AUEC_{0-t(Ret\%)}$(%*hr)	641.07	627.02	1.02	(0.98, 1.06)	(0.98, 1.06)
$E_{max\ (Ret\%)}$(%)	2.17	2.12	1.02	(0.99, 1.06)	(0.98, 1.07)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
Study EPOE-12-02

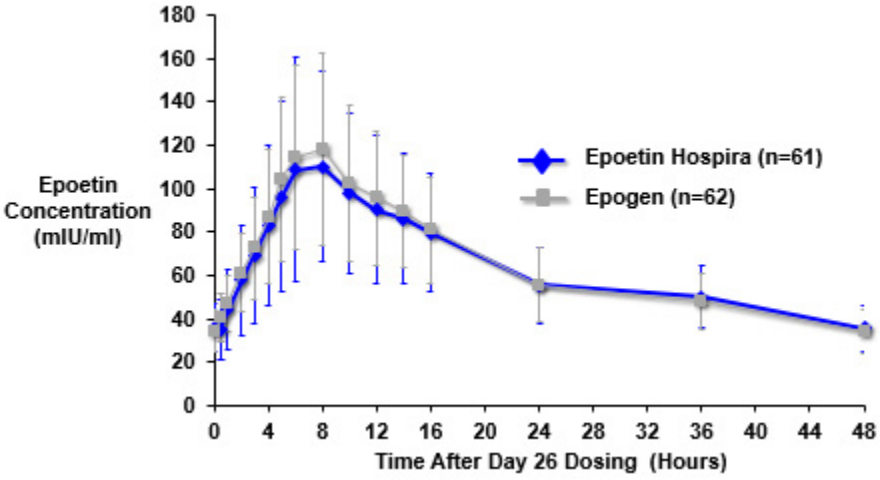
6.1.4. Multiple-Dose PK/PD Results (EPOE-14-01)

Multiple-Dose Pharmacokinetics

Original Analysis

In multiple-dose study EPOE-14-01, mean (± SD) epoetin concentration-time profiles were similar between the Epoetin Hospira and Epogen treatment groups on Day 26 (Figure 34).

Figure 34. Multiple Dose Pharmacokinetics: Mean (± SD) Serum Epoetin Concentration Profiles over Time on Day 26 after Subcutaneous Administration of 100 U/kg TIW for 4 Weeks of Epoetin Hospira or Epogen to Healthy Male Subjects in the Multiple Dose PK/PD Study (Pharmacokinetic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing was at Time 0 on Day 26.
Study EPOE-14-01

The PK population on which the PK analysis was conducted consisted of subjects who had sufficient data to calculate the primary PK parameters (AUC_{0-48} and C_{max}) and excluded subjects who had a positive ADA. PK similarity between Epoetin Hospira and Epogen following multiple-dose administration was demonstrated based on 90% CIs for the GMRs (Epoetin Hospira/Epogen) being contained within the prospectively defined acceptance limits of 0.80 – 1.25 for both the AUC_{0-48} and C_{max} (Table 23), and was supported by sensitivity analysis conducted in subjects who received at least one dose of study drug (data included in the BLA).

Table 23. Pharmacokinetic Evaluation in the Multiple Dose PK/PD Study (Original Analysis, Pharmacokinetic Population)

Parameter	Statistic	Epoetin Hospira (N = 61)	Epogen (N = 62)	Ratio	90% CI for Ratio
AUC_{0-48} (mIU*hr/mL)	Geometric Mean	2917.85	2995.71	--	--
	LS Mean (SE)	2917.85 (1.036)	2995.71 (1.036)	0.974	(0.896, 1.059)
C_{max} (mIU/mL)	Geometric Mean	111.47	118.83	--	--
	LS Mean (SE)	111.47 (1.049)	118.83 (1.049)	0.938	(0.839, 1.049)

Study EPOE-14-01

Supplemental Analysis

A supplemental immunogenicity assessment was performed using updated validated stringent in-study cutpoints to identify ADA-positive subjects. Based on these supplemental

immunogenicity results, a supplemental analysis for the PK primary endpoints was conducted which excluded these ADA-positive subjects from the PK population. The results of the supplemental analysis for the PK primary endpoints are shown in Table 24.

The GMR for AUC_{0-48} was 0.968, with the 90% CI (0.888, 1.056) completely contained within the acceptance limits of 0.80 – 1.25. The GMR for C_{max} was 0.926, with the 90% CI (0.824, 1.040) completely contained within the acceptance limits of 0.80 - 1.25. These results are consistent with those observed for the PK Population.

Table 24. Pharmacokinetic Evaluation in the Multiple Dose PK/PD Study (Supplemental Analysis, Pharmacokinetic Population)

Parameter	Statistic	Epoetin Hospira (N = 57)	Epogen (N = 59)	Ratio	90% CI for Ratio
AUC_{0-48} (mIU*hr/mL)	Geometric Mean	2915.47	3010.58	--	--
	LS Mean (SE)	2915.47 (1.038)	3010.58 (1.037)	0.968	(0.888, 1.056)
C_{max} (mIU/mL)	Geometric Mean	110.60	119.47	--	--
	LS Mean (SE)	110.60 (1.051)	119.47 (1.050)	0.926	(0.824, 1.040)

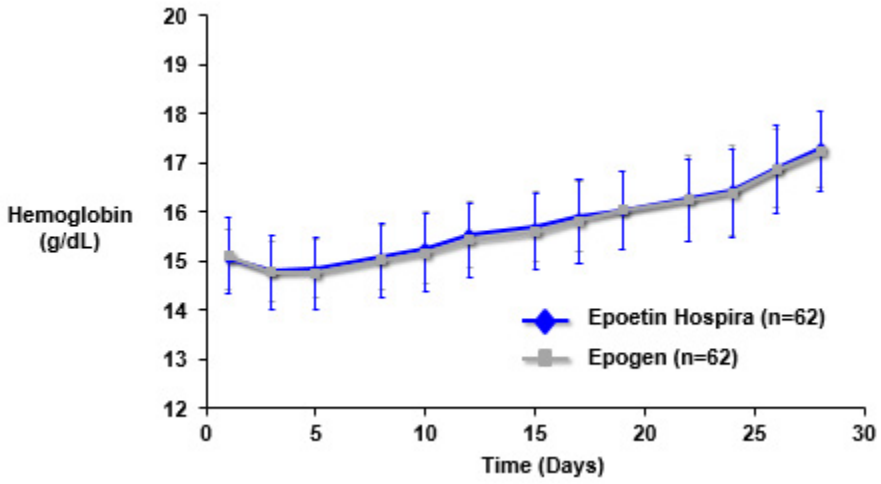
Study EPOE-14-01

Multiple Dose Pharmacodynamics

Original Analysis

Hemoglobin-time profiles from baseline through Day 28 were similar between the Epoetin Hospira and Epogen treatment groups (Figure 35). As noted above, Hb is a well-established PD marker reflective of the known mechanism of action of epoetin on erythropoietic response and a measure of therapeutic effect, and is therefore an appropriate PD parameter to follow epoetin-stimulated erythropoiesis. The Hb response takes longer to manifest compared to reticulocyte count (Cheung et al., 2001; Ramakrishnan et al., 2004; Sorgel et al., 2009), therefore Hb response and consistency over time is best evaluated in a multiple-dose study.

Figure 35. Multiple Dose Pharmacodynamics: Mean (± SD) Hemoglobin over Time Profile after Multiple-Dose Subcutaneous Administration of Epoetin Hospira or Epogen in the Multiple Dose PK/PD Study (Pharmacodynamic Population)



Values are shown as mean with bars representing ± 1 SD. Dosing at Time 0.
Study EPOE-14-01

The PD population on which the PD analysis was conducted consisted of subjects who had sufficient data to calculate the primary PD variable ($AUEC_{Hb}$) and excluded subjects who had a positive ADA. Pharmacodynamic similarity between Epoetin Hospira and Epogen following multiple-dose administration was demonstrated based on the FDA-requested 90% CI for GMR (Epoetin Hospira/Epogen) for $AUEC_{Hb}$ being contained within the prospectively-defined acceptance limits of 0.965 – 1.035 (Table 25), and was supported by sensitivity analysis in subjects who received at least one dose of study drug (data included in the BLA). The PD equivalence margin was assessed considering the range of Hb values specified at entry of 13.0-15.5 g/dL (midpoint 14.2 g/dL) and the established Hb equivalence margin used in the comparative safety and efficacy studies of ± 0.5 g/ dL. The corresponding equivalence acceptance range is calculated as a percent as ± (0.5/14.2) x 100 = ±3.5%. In this analysis, an analysis of covariance (ANCOVA) model was used, with baseline Hb as a covariate and treatment group as a factor. Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated based on pre-specified 95% CI for GMRs (Table 25).

During the BLA review, FDA requested analyses on the E_{max} for Hb. The GMR (Epoetin Hospira/Epogen) for E_{max} was 1.006 with a 90% CI of (0.995, 1.017).

Table 25. Pharmacodynamic Evaluation in the Multiple Dose PK/PD Study (Original Analysis, Pharmacodynamic Population)

Parameter	Statistic	Epoetin Hospira (N = 62)	Epogen (N = 62)	Ratio	90% CI for Ratio*	95% CI for Ratio**
$AUEC_{Hb}$ (g*hr/dL)	Geometric Mean	10238.11	10199.66	--		--
	LS Mean (SE)	10251.11 (1.004)	10186.73 (1.004)	1.006	(0.998, 1.015)	(0.996, 1.016)

*90% CI requested by FDA during BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
EPOE-14-01

Supplemental Analysis

A supplemental immunogenicity assessment was performed using updated validated stringent in-study cutpoints to identify ADA-positive subjects. Based on these supplemental immunogenicity results, a supplemental analysis for the PD primary endpoints was conducted to exclude ADA-positive subjects from the PD population. The results of the supplemental analysis for the PD primary endpoints are shown in Table 26.

The GMR for $AUEC_{Hb}$ for the supplemental analysis was 1.005, with the FDA-requested 90% CI (0.997, 1.014) completely contained within the acceptance limits of 0.965-1.035, consistent with the results of the primary PD evaluation. Pharmacodynamic similarity between Epoetin Hospira and Epogen was also demonstrated based on the pre-specified 95% CI for GMR ($AUEC_{Hb}$: 0.995, 1.016).

During the BLA review, FDA requested analyses on the E_{max} for Hb for the supplemental analysis. The GMR (Epoetin Hospira/Epogen) for E_{max} for the supplemental analysis was 1.006 with a 90% CI of (0.995, 1.017).

Table 26. Pharmacodynamic Evaluation in the Multiple Dose PK/PD Study (Supplemental Analysis, Pharmacodynamic Population)

Parameter	Statistic	Epoetin Hospira (N = 58)	Epogen (N = 59)	Ratio	90% CI for Ratio*	95% CI for Ratio**
$AUEC_{Hb}$ (g*hr/dL)	Geometric Mean	10221.59	10177.16	--		--
	LS Mean (SE)	10227.19 (1.004)	10171.68 (1.004)	1.005	(0.997, 1.014)	(0.995, 1.016)

*90% CI requested by FDA
**95% CI pre-specified in Sponsor analysis
EPOE-14-01

6.2. Clinical Efficacy

6.2.1. Summary of Efficacy

In 2 randomized, double-blind, comparative clinical studies, similar efficacy was observed between Epoetin Hospira and the Epogen reference product when administered SC or IV in subjects with renal anemia, supporting demonstration of biosimilarity.

Equivalence was established using the co-primary endpoints of mean weekly Hb and mean weekly dose during the last 4 weeks of treatment under SC and IV conditions. As agreed to by the FDA during initial discussions and stated in the protocols, equivalence was to be established if the Sponsor-pre-specified 95% CIs for the difference between Epoetin Hospira and Epogen in mean weekly Hb and mean weekly dose during the last 4 weeks of the nominal treatment period (defined as the 16-week Maintenance Period in Study EPOE-10-13 [SC] and the 24-week Treatment Period in Study EPOE-10-01 [IV]) were within the pre-specified equivalence limits of ± 0.5 g/dL and ± 45 U/kg/week, respectively. Subsequently, during the 2017 BLA review the FDA requested that 90% CIs be used. Both the 90% CIs and the 95% CIs are provided in the displays for clarity.

- The 90% CIs for the difference in mean weekly Hb during the last 4 weeks of treatment between Epoetin Hospira and Epogen were (-0.13, 0.21) for SC administration and (-0.22, -0.01) for IV administration, both within the pre-specified equivalence limits of ± 0.5 g/ dL. The Sponsor pre-specified 95% CIs for both SC and IV administration were also within the pre-specified equivalence limits.
- The 90% CIs for the difference in mean weekly dose per kg body weight during the last 4 weeks of treatment between Epoetin Hospira and Epogen were (-12.54, 7.85) for SC administration and (-8.67, 9.40) for IV administration, both within the pre-specified equivalence limits of ± 45 U/kg/week. The Sponsor pre-specified 95% CIs for both SC and IV administration were also within the pre-specified equivalence limits.

Equivalence was thus established using the Sponsor-pre-specified 95% CI and the FDA-requested 90% CI for the co-primary endpoints. Results of sensitivity analyses on the co-primary endpoints and key secondary endpoints, including assessment of subjects requiring transfusions, underscore the robust nature of the efficacy results. Subgroup analysis demonstrated no clinically meaningful effects of intrinsic or extrinsic factors on the results for the co-primary endpoints.

6.2.2. Study Design

The study designs of the two comparative efficacy and safety studies in subjects with CKD are presented in Table 27.

The two randomized clinical studies, EPOE-10-13 and EPOE-10-01, were conducted in the United States by 52 Principal Investigators at 68 clinical sites and by 78 Principal Investigators at 95 clinical sites, respectively. Of note, a single investigator may have overseen more than one clinical site within a study or have participated in both studies. During clinical conduct, the sponsor closed clinical sites overseen by 7 principal investigators across the clinical program due to GCP non-compliance. The proportion of the ITT Population impacted by closing these clinical investigator sites is approximately 9%. Sensitivity analysis (Section 6.2.5.2) removing subjects from the closed sites from the ITT Population are concordant with the primary ITT analysis conclusions in support of a demonstration of biosimilarity between Epoetin Hospira and Epogen. Overall, there is no impact of closed sites on the overall conclusions of the studies.

Table 27. Summary of Comparative Clinical Efficacy and Safety Studies in Epoetin Hospira Clinical Development Program

	Subcutaneous Comparative Safety and Efficacy Study (EPOE-10-13) (N = 320)	Intravenous Comparative Safety and Efficacy Study (EPOE-10-01) (N = 612)
Subjects	CKD on HD	CKD on HD
Study design	Multicenter (68 in US), double-blind, randomized, active-control	Multicenter (95 in US, 1 in PR), double-blind, randomized, active-control
Dosing	Dose Stabilization Period: SC dose stabilization x 12-18 wks for subjects treated with IV Epogen at Screening[a] Maintenance Period: SC 1 to 3 times/wk to maintain Hb between 9.0-11.0 g/dL for 16 wks	IV 1 to 3 times/wk to maintain Hb between 9.0-11.0 g/dL for 24 wks during Treatment Period
Co-primary or Primary Endpoint(s)	Mean weekly Hb **and** mean weekly dose per kg body weight during last 4 weeks of the Maintenance Period	Mean weekly Hb **and** mean weekly dose per kg body weight during last 4 weeks of the Treatment Period
Other Endpoints	Immunogenicity, Safety	Immunogenicity, Safety

CKD = chronic kidney disease; Hb = hemoglobin; HD = hemodialysis; IV = intravenous; PR = Puerto Rico; SC= subcutaneous; TEAE = treatment-emergent adverse event; wk = week

a. Subjects treated with IV Epogen at Screening received study drug by SC injection with an initial 20-30% dose reduction from the IV weekly dose the subject received during the last week of the up-to-4-week Screening Period. Subjects were treated for 12 to 18 weeks in the Dose Stabilization Period to achieve at least 4 weeks of protocol-defined optimal stable dosing. All subjects must have been optimally titrated and on stable dose to qualify for entry into the Maintenance Period. Subjects who had been on SC treatment at the time of Screening and had demonstrated protocol-defined optimal stable dosing were randomized into the Dose Stabilization Period, received study drug assignment, and then proceeded directly to randomization into the Maintenance Period.

Subcutaneous Efficacy and Safety Study (EPOE-10-13)

EPOE-10-13 was a multicenter, double-blind, randomized, active-controlled, parallel group, comparative efficacy and safety study of subjects with CKD requiring HD and receiving epoetin maintenance treatment prior to enrollment. Eligible subjects (described in Section 6.2.2.1) were randomized (1:1) to Epoetin Hospira or Epogen in a Dose Stabilization Period and required to have a stable SC dosing before a second randomization (1:1 to Epoetin Hospira or Epogen) into the Maintenance Period (Figure 36).

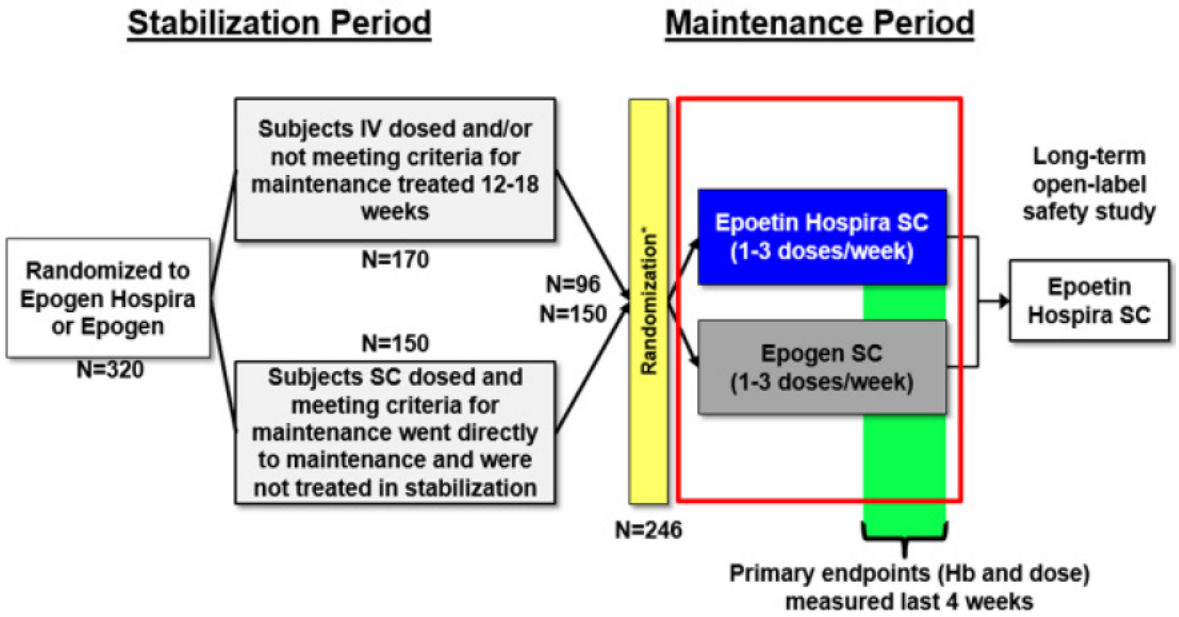
The inclusion of a Dose Stabilization Period into the study design provided a mechanism to transition subjects who were maintained on the more prevalent IV route of epoetin treatment prior to study participation to a stable SC epoetin regimen. In addition, it provided a mechanism to allow subjects who had been on SC treatment, but had not yet achieved a stable regimen, to establish a stable dosing regimen. Subjects who had been on SC treatment at the time of Screening and had demonstrated protocol-defined optimal stable dosing were

randomized into the Maintenance Period without any follow up or treatment in the Stabilization Period.

For subjects who had been on IV Epogen prior to study enrollment, the SC dose was reduced an initial 20-30% from the IV weekly dose they received during the last week of the up-to-4-week Screening Period. Subjects were then randomized into the 12- to 18-week Dose Stabilization Period to achieve at least 4 weeks of optimal stable dosing, which was required to qualify for entry into the Maintenance Period.

Subjects were treated for up to 16 weeks in the Maintenance Period. After completing the Maintenance Period, all subjects had the opportunity to enter an open-label long-term safety study (LTSS) EPOE-11-04 (SC), and be treated with Epoetin Hospira for up to an additional 48 weeks. Subjects who discontinued from randomized study treatment during the Maintenance Period were also eligible to enter the LTSS.

Figure 36. Subcutaneous Comparative Efficacy and Safety Study Schematic (Study EPOE-10-13)



• Patients were a) stable SC for ≥ 4 weeks at screening, b) stabilized on Epoetin Hospira SC over 12-18 wks, or c) stabilized on Epogen SC over 12-18 wks

Of the 74 subjects who were randomized into the Stabilization Period, but were not randomized into the Maintenance Period, 4 subjects were never treated in the Stabilization Period, 33 subjects were treated with Epoetin Hospira in the Stabilization Period, and 37 subjects were treated with Epogen in the Stabilization Period. Thus, the number of subjects who discontinued from the study during the Stabilization Period was comparable between the Epoetin Hospira and Epogen treatment groups.

During the study, the dose of study drug was evaluated for adjustment on a regular basis (i.e., at least every week) to maintain the Hb value within a range of 9.0 to 11.0 g/dL. Adjustments to dose for study treatment were allowed in line with the approved Epogen US Package Insert (Epogen PI, 2014).

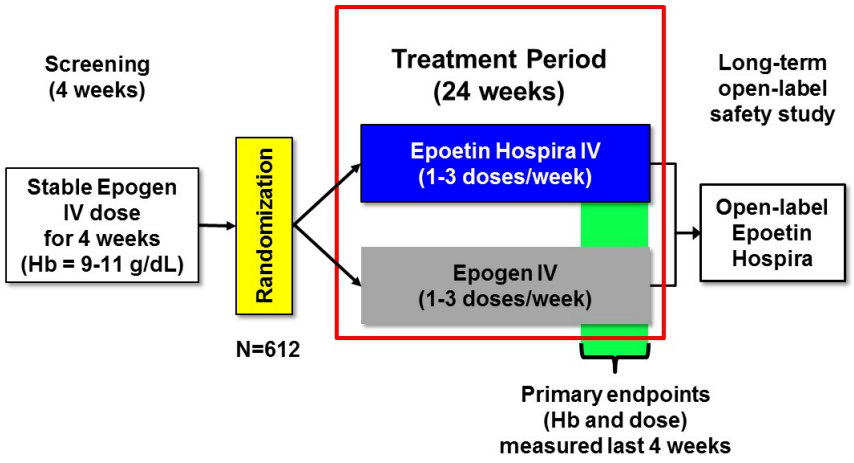
Intravenous Efficacy and Safety Study (EPOE-10-01)

Study EPOE-10-01 was a multicenter, double-blind, randomized, active-controlled, parallel group, comparative efficacy and safety study of subjects with CKD requiring HD and receiving IV epoetin maintenance treatment prior to enrollment. Because subjects were already receiving IV treatment no stabilization period was necessary, though they did need to be on a stable weekly dose prior to enrollment in the study.

Eligible subjects (identical to those enrolled in Study EPOE-10-13 [SC] described in Section 6.2.2.1) were randomized in a 1:1 ratio to either Epoetin Hospira or Epogen as IV bolus injections administered 1 to 3 times per week at the same stable weekly dose that the subject received during the last week of the up-to-4-week Screening Period (Figure 37). Subjects were treated for up to 24 weeks in the Treatment Period. During the study, investigators adjusted the dose, as needed, to maintain subjects' Hb within a range of 9.0 to 11.0 g/dL, using the same guidelines as those followed in Study EPOE-10-13 (SC).

After completing the Treatment Period, all subjects had the opportunity to enter LTSS EPOE-11-03 (IV) and be treated with Epoetin Hospira for up to an additional 48 weeks. Subjects who discontinued from the randomized study drug during the Treatment Period were also eligible to enter the LTSS.

Figure 37. Intravenous Comparative Efficacy and Safety Study Schematic (Study EPOE-10-01)



Note: Intravenous Comparative Efficacy and Safety Study did not have a Stabilization Period; Treatment Period defined by red box.

6.2.2.1. Study Population

The study population for Studies EPOE-10-13 (SC) and EPOE-10-01 (IV) consisted of male and non-pregnant female subjects with CKD on HD and with anemia who:

- were aged 18 to 80 years old (inclusive),

- were on stable IV or SC Epogen treatment 1 to 3 times per week for at least 4 weeks prior to randomization,
- had stable Hb (mean between 9.0 and 11.0 g/dL) for 4 weeks prior to randomization,
- were on stable, adequate dialysis for at least 12 weeks prior to randomization,
- had adequate iron stores defined as ferritin >100 mcg/L and transferrin saturation (TSAT) >20% prior to randomization,
- required maintenance doses of Epogen no greater than 600 U/kg/week, and
- received no long-acting epoetin analogues for at least 12 weeks prior to randomization.

6.2.2.2. Study Endpoints

Co-Primary Efficacy Endpoints

For Studies EPOE-10-13 (SC) and EPOE-10-01 (IV), the co-primary efficacy endpoints, calculated from Hb levels and dose data collected during the last 4 weeks of treatment with Epoetin Hospira and Epogen, were:

- Difference between treatments (Epoetin Hospira and Epogen) in mean weekly Hb level during the last 4 weeks of the double-blind treatment period
- Difference between treatments (Epoetin Hospira and Epogen) in mean weekly dose per kg body weight during the last 4 weeks of the double-blind treatment period

Hemoglobin is a well-characterized and well-established measure for ESA product development, and indeed, attainment and maintenance of Hb level within the target range of 9.0 to 11.0 g/dL is the therapeutic target for epoetin administration. Thus, Hb level is an appropriate co-primary endpoint. For this reason, both Study EPOE-10-13 (SC) and Study EPOE-10-01 (IV) were designed to enroll subjects with CKD on HD who were already receiving epoetin, and the goal of epoetin administration in these studies was maintenance of Hb within the target range.

For the difference between the mean weekly Hb levels, a pre-defined equivalence margin of ± 0.5 g/dL was used.

For the difference between the mean weekly dose per kg of body weight, the acceptance pre-defined margin of ± 45 U/kg/week was used.

Because the treatment goal is to maintain Hb levels within the desired therapeutic range using the epoetin dose, comparison of Epoetin Hospira and Epogen for both dose and the resulting Hb levels are the most appropriate efficacy measures to perform comparative efficacy assessments between the two products. The use of these two endpoints is a well-characterized standard method of assessing comparative efficacy of proposed biosimilar erythropoiesis-stimulating agents (ESAs) and reference products (Wizemann et al., 2008; Krivoshiev et al., 2008; Krivoshiev et al., 2010).

Justification of Margins

In a maintenance therapy setting, the treatment goal is to maintain Hb within a target therapeutic range by adjusting the EPO dose. As such, the traditional method of establishing an equivalence margin based upon the treatment difference between active and placebo may not be meaningful. For the epoetin program, establishment of the margins was based upon determining a clinically meaningful difference for Hb and dose.

Rationale for Selection of Hemoglobin Equivalence Margin of ± 0.5 g/dL

For the difference between the mean weekly Hb levels, an equivalence margin of ± 0.5 g/dL was used. The rationale for selecting this equivalence margin was based on previous studies with Eprex® (Janssen-Cilag), Aranesp® (Amgen), EU Binocrit® (Sandoz), and EU Retacrit® (Hospira UK Limited) (Wizemann et al., 2008) establishing equivalence and other literature. High intra-individual variability of Hb levels in patients with renal anemia is described in the literature. One observational study in 987 epoetin-treated HD patients found that the Hb variability ranges that encompassed 90% of patients using 1-month, 3-month, and 6-month rolling averages were 4.4 g/dL, 3.7 g/dL, and 3.2 g/dL, respectively (Berns et al., 2003). Fewer than 50% of these patients had Hb variability values within a range of 1.0 g/dL recommended by the National Kidney Foundation-Kidney Disease Outcome Quality Initiative (NKF-KDOQI), even when a 6-month rolling average was applied. Another study evaluated Hb variability in 48,133 patients with end stage renal failure (Lacson et al., 2003). The average individual patient was calculated to have an expected fluctuation of ± 1.4 g/dL in three-month rolling average Hb levels per year. Therefore, the data from the literature indicate that even "stable" patients with renal anemia on stable epoetin doses experience intra-individual fluctuations in Hb of approximately ± 1 g/dL. Thus, an equivalence margin of ± 0.5 g/dL is considered relevant to demonstrate the equivalence of the two epoetin products.

Rationale for Selection of Dose Acceptance Margin of ± 45 U/kg/Week

For the difference between the mean weekly dose per kg of body weight, an equivalence margin of ± 45 U/kg/week was used. The rationale for selecting this equivalence margin was based on the fact that 45 U/kg/week is a no effect dose (Eschbach et al., 1987; FDA, SBA Epogen, 1989; Dynepo, EMA Report, 2004). An acceptance margin of ± 45 U/kg/week has been used to demonstrate therapeutic acceptance of Eprex and EU-approved Retacrit as part of the establishment of biosimilarity of the latter in Europe (Wizemann et al., 2008; Krivoshiev et al., 2008; Krivoshiev et al., 2010). Also, the Epogen US Package Insert (Epogen PI, 2014) recommends 25% or greater dose change when modifying dose, corresponding to a dose change of at least ± 37.5 to ± 75 U/kg/week. Taken together, the ± 45 U/kg/week is considered relevant to demonstrate the equivalence of the two epoetin products.

Additionally, the Normal Hematocrit Study (NHS, Besarab et al., 1998) (Table 28) demonstrated a difference in outcome with the two target Hct treatment groups and provides additional support for the relevance of the established margins. For corresponding Hb, the

upper limit of 0.5 g/dL represents 13% of the clinically meaningful difference seen (4 g/dL) in the NHS. For dose, the upper limit of 45 U/kg/week represents 15% of the clinically meaningful difference (300 U/kg/week) seen in the NHS.

Table 28. Design of the Normal Hematocrit Study

Parameter	Therapeutic Category		Difference
	Low (N=615)	Normal (N=618)	
Target Hct	30	42	12%
Corresponding Hb (g/dL)	10	14	4 g/dL
Dose (U/kg/week)	150	450	300 U/kg/week

Reference: Besarab et al. 1998

Secondary Efficacy Endpoints

Multiple secondary efficacy endpoints were assessed for comparative differences between Epoetin Hospira and Epogen treatments. Those conducted on the Intent-to-Treat (ITT) population for which results are presented in this Briefing Document include:

- Mean weekly Hb level over the duration of the nominal treatment period (defined as the 16-week Maintenance Period in Study EPOE-10-13 [SC] and/or the 24-week Treatment Period in Study EPOE-10-01 [IV])
- Mean weekly dose per kg body weight delivered over the duration of the nominal treatment period
- Proportion of subjects with a weekly mean Hb level within the target range (9.0 11.0 g/dL) at weeks 16 (Study EPOE-10-13 [SC]) or week 24 (Study EPOE-10-01 [IV]) of the Maintenance Period
- Incidence of subjects receiving blood transfusions in the Maintenance Period

6.2.3. Subject Disposition

The majority of subjects who participated in Study EPOE-10-13 (SC) or Study EPOE-10-01 (IV) completed the study (Table 29).

Table 29. Summary of Subject Disposition

	Number of Subjects (%)			
	Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)		Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)	
	Epoetin Hospira (N = 124)	Epogen (N = 122)	Epoetin Hospira (N = 306)	Epogen (N = 306)
Treated	123 (99.2)	121 (99.2)	300 (98.0)	305 (99.7)
Completed study	106 (85.5)	105 (86.1)	252 (82.4)	259 (84.6)
Discontinued study	18 (14.5)	17 (13.9)	54 (17.6)	47 (15.4)
Primary Reason for Study Discontinuation				
Adverse event	3 (2.4)	2 (1.6)	8 (2.6)	8 (2.6)
Other*	15 (12.1)	15 (12.3)	46 (15.0)	39 (12.7)
Primary Reason for Study Drug Discontinuation				
Adverse Event	6 (4.8)	2 (1.6)	9 (2.9)	10 (3.3)
Other*	13 (10.5)	12 (9.8)	41 (13.3)	40 (13.1)
Non-Study ESA	5 (4.0)	8 (6.6)	42 (13.7)	43 (14.1)

*Other includes: Withdrawal of consent; Randomization error; Lost to Follow-up, Protocol deviation/ violation, and Physician decision

6.2.4. Demographics and Baseline Characteristics

The demographics and baseline characteristics of subjects randomized to Study EPOE-10-13 (SC) and Study EPOE-10-01 (IV) (Table 30) are balanced between the treatment groups and representative of the general population of CKD patients on HD (USDS, 2013). The majority of subjects (~80%) reported the primary cause of CKD as either diabetes or hypertension. The treatment groups were also comparable based on subjects' mean baseline Hb (~10.4 g/dL), mean weekly dose (101 to 103 U/kg/week), and dose frequency (~38% reporting once per week, ~15% reporting twice a week, and ~47% reporting three times per week).

Table 30. Summary of Subject Demographics and Baseline Characteristics

Parameter	Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)		Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)	
	Epoetin Hospira	Epogen	Epoetin Hospira	Epogen
Sex, n (%)				
N	124	122	306	306
Female	60 (48.4)	66 (54.1)	146 (47.7)	131 (42.8)
Male	64 (51.6)	56 (45.9)	160 (52.3)	175 (57.2)
Race[a] Group, n (%)				
N	124	122	306	306
White	69 (55.6)	59 (48.4)	142 (46.4)	151 (49.3)
Black or African-American	50 (40.3)	59 (48.4)	149 (48.7)	127 (41.5)
Other	5 (4.0)	4 (3.2)	15 (4.9)	27 (8.7)
Missing	0	0	0	1 (0.3)
Age (years)				
N	124	122	306	306
Mean (SD)	56.95 (11.929)	56.94 (13.484)	55.32 (13.057)	57.35 (11.440)
Median	58.00	60.00	57.00	58.00
Min, Max	25.0, 80.0	24.0, 79.0	21.0, 78.0	25.0, 80.0
Body Mass Index (kg/m^2)				
N	124	122	305	304
Mean (SD)	30.02 (7.025)	30.73 (7.927)	30.98 (8.488)	30.64 (7.945)
Median	29.61	29.46	29.80	29.45
Min, Max	17.6, 51.4	16.9, 56.7	15.4, 89.8	17.1, 96.3
Primary Cause of Chronic Kidney Disease, n (%)				
N	124	122	306	306
Diabetes	56 (45.2)	41 (33.6)	145 (47.4)	151 (49.3)
Hypertension	43 (34.7)	58 (47.5)	105 (34.3)	85 (27.8)
Nephropathies	13 (10.5)	16 (13.1)	36 (11.8)	44 (14.4)
Congenital renal disease	5 (4.0)	3 (2.5)	6 (2.0)	10 (3.3)
Other	7 (5.6)	4 (3.3)	10 (3.3)	12 (3.9)
Unknown	0	0	3 (1.0)	3 (1.0)
Time from Start of Regular Dialysis to Randomization (months)				
N	124	122	305	305
Mean (SD)	53.54 (52.248)	57.93 (41.612)	51.49 (50.938)	53.56 (50.941)
Median	41.00	48.50	35.00	38.00
Min, Max	2.0, 336.0	3.0, 187.0	4.0, 434.0	3.0, 351.0

Parameter	Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)		Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)	
	Epoetin Hospira	Epogen	Epoetin Hospira	Epogen
Baseline Dose by Weight (U/kg/week)				
N	123	122	305	304
Mean (SD)	93.55 (111.516)	86.33 (83.165)	105.98 (97.508)	107.64 (103.936)
Median	56.76	53.00	73.70	76.73
Min, Max	3.2, 644.5	4.9, 383.0	2.6, 582.9	1.3, 675.6
Baseline Dose Frequency (per week) [n(%)]				
1	90 (72.6)	93 (76.2)	72 (23.5)	74 (24.2)
2	15 (12.1)	11 (9.0)	50 (16.3)	53 (17.3)
3	18 (14.5)	18 (14.8)	183 (59.8)	179 (58.5)
Missing	1 (0.8)	0	0	0
Baseline Hemoglobin (g/dL)				
n	124	122	305	306
Mean (SD)	10.36 (0.777)	10.27 (0.773)	10.43 (0.769)	10.43 (0.712)
Median	10.30	10.40	10.40	10.50
Min, Max	8.2, 12.8	7.4, 12.1	8.3, 13.6	8.6, 12.6
Baseline Ferritin (ng/mL)				
n	124	122	306	306
Mean (SD)	981.8 (413.16)	928.8 (398.75)	925.2 (443.20)	937.1 (417.87)
Median	971.0	922.5	879.5	901.5
Min, Max	125, 2085	82, 2026	105, 4704	209, 2814
Baseline TSAT (%)				
n	124	122	306	306
Mean (SD)	35.8 (13.33)	34.4 (14.50)	34.2 (11.66)	33.3 (10.85)
Median	32.0	31.0	33.0	31.0
Min, Max	16, 89	8, 96	11, 91	9, 81
CRP (mg/dL)				
n	124	122	306	306
Mean (SD)	0.954 (1.3474)	1.225 (2.0918)	1.055 (1.913)	1.021 (1.480)
Median	0.450	0.565	0.510	0.540
Min, Max	0.02, 7.37	0.03, 13.81	0.02, 19.85	0.03, 12.70

CRP, C-reactive protein, TSAT, transferrin saturation.
a. Since subjects can select multiple races, the percentages may not add up to 100.
Note: Data for all parameters are based on Intent-to-Treat Population.

6.2.5. Efficacy Results

6.2.5.1. Co-Primary Endpoints

Both comparative studies EPOE-10-13 (SC) and EPOE-10-01 (IV) met their co-primary endpoints for efficacy by demonstrating equivalence between Epoetin Hospira and the Epogen reference product, when administered SC or IV, in maintaining mean weekly Hb level and in the administered mean weekly dose per kg body weight to maintain Hb within the target range of 9.0 to 11.0 g/dL (Table 31).

The FDA-requested 90% CIs for the difference in mean weekly Hb level during the last 4 weeks of the nominal treatment period between Epoetin Hospira and Epogen were (-0.13, 0.21) for SC administration and (-0.22, -0.01) for IV administration, both within the pre-specified equivalence limits of ± 0.5 g/dL. The FDA-requested 90% CIs for the difference in mean weekly dose per kg body weight during the last 4 weeks of the nominal treatment period between Epoetin Hospira and Epogen were (-12.54, 7.85) for SC administration and (-8.67, 9.40) for IV administration, both within the pre-specified equivalence limits of ± 45 U/kg/week. The Sponsor pre-specified 95% CIs for the difference in mean weekly Hb level and mean weekly dose per kg body weight during the last 4 weeks of the nominal treatment period were also contained with the respective pre-specified equivalence limits.

Table 31. Mean Weekly Hemoglobin and Mean Weekly Dose per Kilogram Body Weight during the Last 4 Weeks of the Nominal Treatment Period (Intent-to-Treat Population)

Parameter		Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)			Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)		
	Statistic	Epoetin Hospira (N=124)	Epogen (N=122)	Difference	Epoetin Hospira (N=306)	Epogen (N=306)	Difference
Mean Weekly Hb (g/dL)	LS Mean (SE)	10.16 (0.073)	10.12 (0.074)	0.04 (0.104)	10.17 (0.047)	10.28 (0.047)	-0.12 (0.066)
	90% CI*			(-0.13, 0.21)[a]			(-0.22, -0.01)[a]
	95% CI**			(-0.17, 0.24)			(-0.25, 0.01)
Mean Weekly Dose (U/kg)	LS Mean (SE)	79.57 (4.356)	81.91 (4.373)	-2.34 (6.175)	90.16 (3.874)	89.79 (3.880)	0.37 (5.483)
	90%CI*			(-12.54, 7.85)[b]			(-8.67, 9.40)[b]
	95% CI**			(-14.51, 9.82)			(-10.40, 11.13)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
a. Equivalence is concluded if the 90% confidence interval of the LS Mean of the difference is contained within -0.5 and 0.5 g/dL.
b. Equivalence is concluded if the 90% confidence interval of the LS Mean of the difference is contained within -45 and 45 U/kg/week.
Note: LS Means and confidence intervals come from an ANCOVA model with fixed effect of treatment and baseline as a covariate.
Note: Using a hierarchical test strategy, equivalence of mean weekly Hb level was tested first. If equivalence was concluded, then equivalence of mean weekly dose per kg body weight was tested. If equivalence was concluded for both endpoints, then equivalence in efficacy between Epoetin Hospira and Epogen was concluded.
Note: Nominal treatment period is the 16-week Maintenance Period in Study EPOE-10-13 and/or the 24-week Treatment Period in Study EPOE-10-01

With respect to assessment of the underlying data distribution, both heteroskedasticity and normality assumptions were evaluated for co-primary endpoints. There was no evidence of heteroskedasticity for co-primary endpoints. For the mean weekly Hb level during the last 4 weeks of the Treatment Period, the data did not deviate significantly from the normality assumption. For mean weekly dose per kg body weight during the last 4 weeks of the Treatment Period, the data deviated significantly from the normality assumption. Various data transformation functions were applied and none of them was able to adequately transform the data to meet the normality assumption. Therefore, the primary analysis for mean weekly dose per kg body weight during the last 4 weeks of treatment was conducted on the original scale. Furthermore, the consistency of cumulative distribution for the co-primary endpoints was evaluated (Figure 38 and Figure 39) and indicates that the distributions are not significantly different between treatment groups.

Figure 38. Cumulative Distribution of Hemoglobin during the Last 4 Weeks in the Subcutaneous and Intravenous Comparative Efficacy and Safety Studies

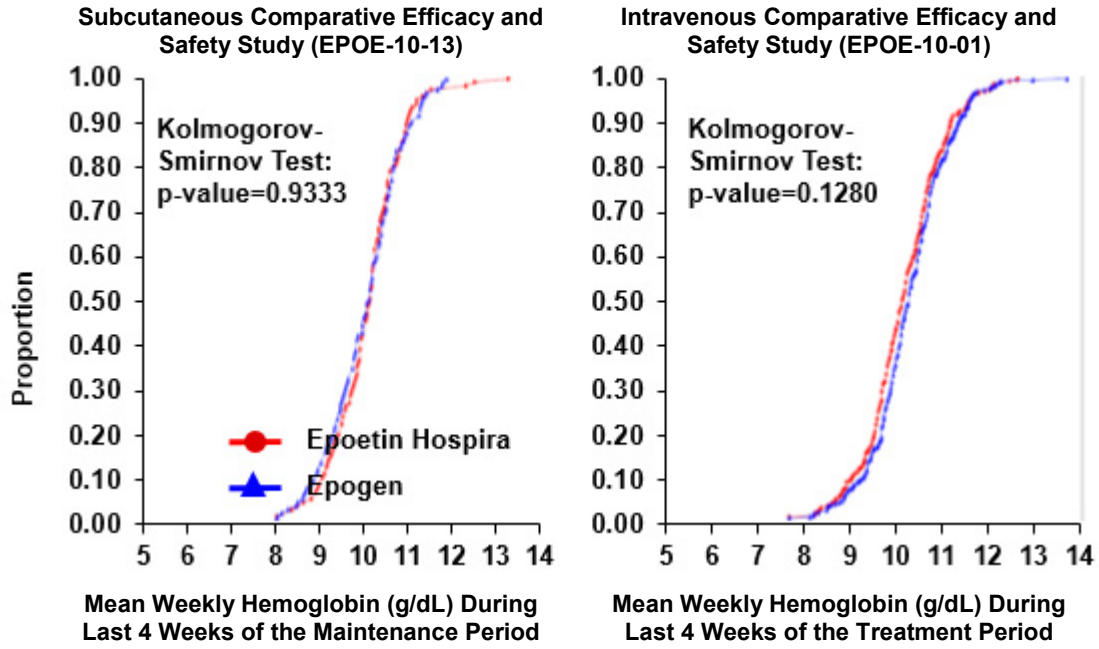
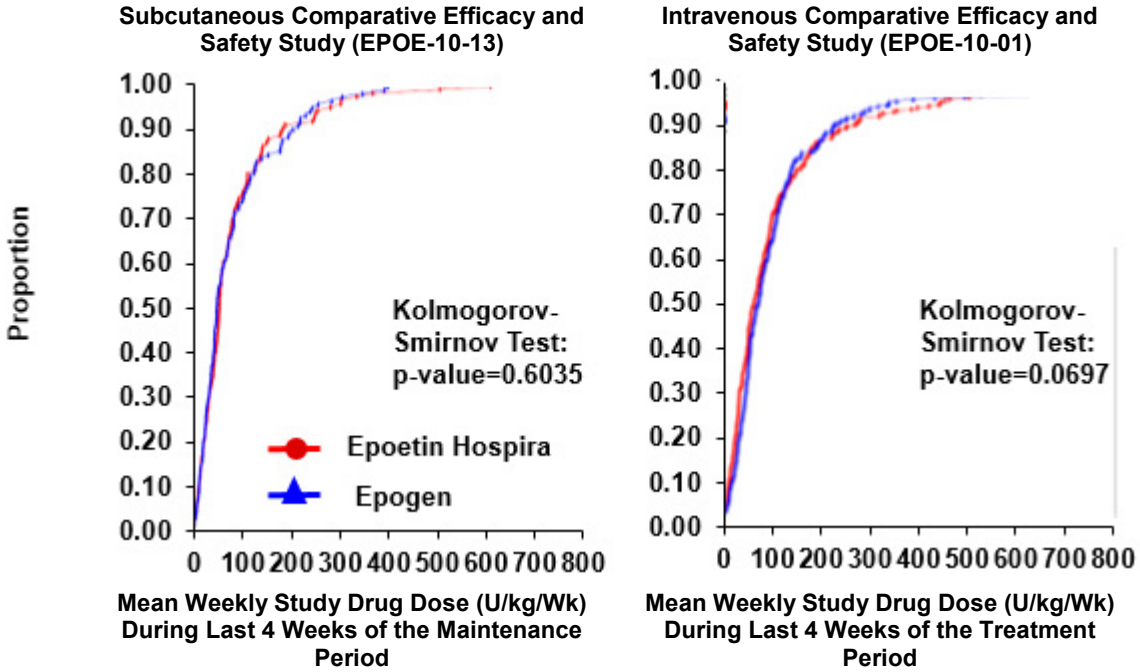


Figure 39. Cumulative Distribution of Dose during the Last 4 Weeks in the Subcutaneous and Intravenous Comparative Efficacy and Safety Studies



6.2.5.2. Sensitivity Analyses on the Co-primary Endpoints

Sensitivity analyses on the co-primary endpoints were performed on various analysis populations as well as using alternative data imputation methodologies for the ITT Population (Table 32).

Table 32. Populations and Alternative Imputation Methods Used for Sensitivity Analyses

Population	Subjects Included
Full Analysis Set (FAS)	All subjects who: (1) received study drug during the Maintenance Period (EPOE-10-13) or the Treatment Period (EPOE-10-01); (2) had both Hb and dose data for the last 4 weeks of the Maintenance Period or Treatment Period, although they may have had the dose held during the last week, and (3) had not discontinued study drug during the last 4 weeks of the Maintenance or Treatment Period.
Modified Full Analysis Set (mFAS)	All subjects who received at least one dose of study drug in the Maintenance Period or Treatment Period and had both Hb and dose data for at least two consecutive weeks in the Maintenance Period or Treatment Period.
Retained Set (RET)	All subjects who: (1) received at least one dose of study drug treatment during the Maintenance Period or Treatment Period; (2) and may or may not have discontinued study drug but stayed on study.
Per Protocol (PP)	Subset of the ITT subjects who had (1) received study drug for ≥ 4 weeks in the Maintenance Period or Treatment Period; (2) ≥4 weeks of Hb data while on study drug during the Maintenance Period or Treatment Period; (3) ≥4 weeks of study drug administration data collected while on study drug during the Maintenance Period or Treatment Period; (4) no important protocol deviation; (5) no use of other ESAs during the last 4 weeks of study drug; and (6) received no packed RBC or whole blood transfusions during study conduct.
ITT Excluding Subjects from Closed Sites	Subset of ITT subjects which excluded subjects from sites closed for GCP non-compliance.

The least square (LS) mean estimate of the difference between the Epoetin Hospira and Epogen treatment groups for the mean weekly Hb and mean weekly dose of epoetin per kg of body weight for last 4 weeks of the Maintenance Period for EPOE-10-13 and for the Treatment Period for EPOE-10-01, as well as the FDA-requested 90% CIs and the Sponsor pre-specified 95% CIs, are shown in Table 33 and Table 34, respectively.

In the Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13), for the PP, FAS, mFAS, RET, and ITT Excluding Subjects from Closed Sites Populations, the LS means of the difference in mean weekly Hb during the last 4 weeks of the Maintenance Period ranged from 0.00 to 0.13 g/dL, with the respective 90% CIs all contained within the acceptance limits of -0.5 and 0.5 g/dL. For these same analysis populations, the LS means of the difference in weekly epoetin dose by body weight during the last 4 weeks of the Treatment Period ranged from -2.38 to 1.63 U/kg/week, with the respective 90% CIs all contained

within the acceptance limits of -45 to 45 U/kg/week. The sensitivity analyses are consistent with and provide robustness to the primary analysis conclusions.

Table 33. Sensitivity Analyses: Difference Between Epoetin Hospira and Epogen in Mean Weekly Hb and Mean Weekly Dose by Body Weight During the Last 4 Weeks of the Maintenance Period in the Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)

Analysis Population	Difference Between Epoetin Hospira and Epogen in Mean Weekly Hemoglobin (g/dL) Level during the Last 4 Weeks of the Maintenance Period			Difference Between Epoetin Hospira and Epogen in Mean Weekly Epoetin Dose (U/kg/Week) during Last 4 Weeks of the Maintenance Period		
	Estimate of Difference LS Mean (SE)	90% CI*	95% CI**	Estimate of Difference LS Mean (SE)	90% CI*	95% CI**
Per Protocol EH: n = 86 EP: n = 92	0.00 (0.118)	(-0.20, 0.19)	(-0.23, 0.23)	1.63 (7.152)	(-10.20, 13.46)	(-12.48, 15.75)
Full Analysis Set EH: n = 71 EP: n = 78	0.12 (0.124)	(-0.08, 0.33)	(-0.12, 0.37)	-1.26 (8.084)	(-14.64, 12.12)	(-17.24, 14.72)
Modified Full Analysis Set EH: n = 122 EP: n = 118	0.07 (0.106)	(-0.11, 0.24)	(-0.14, 0.27)	-1.91 (6.283)	(-12.28, 8.47)	(-14.28, 10.47)
Retained Set EH: n = 105 EP: n = 105	0.13 (0.111)	(-0.06, 0.31)	(-0.09, 0.35)	-2.38 (6.862)	(-13.72, 8.96)	(-15.91, 11.15)
ITT Excluding Closed Sites EH: n = 112 EP: n = 114	0.04 (0.108)	(-0.13, 0.22)	(-0.17, 0.26)	0.76 (5.824)	(-8.86, 10.38)	(-10.72, 12.24)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
Abbreviations: CI = confidence interval; EH = subjects randomized to Epoetin Hospira; EP = subjects randomized to Epogen; LS = least square; n = number; SE = standard error
Note: LS Means and confidence intervals come from an ANCOVA model with fixed effect of Treatment and baseline as a covariate.

In the Intravenous Comparative Efficacy and Safety Study (EPOE-10-01), for the PP, FAS, mFAS, RET, and ITT Excluding Subjects from Closed Sites Populations, the LS means of the difference in mean weekly Hb during the last 4 weeks of the Treatment Period ranged from -0.13 to -0.10 g/dL, with the respective 90% CIs all contained within the acceptance limits of -0.5 and 0.5 g/dL. For these same analysis populations, the LS means of the difference in weekly epoetin dose by body weight during the last 4 weeks of the Treatment Period ranged from -6.48 to 0.80 U/kg/week, with the respective 90% CIs all contained within the acceptance limits of -45 to 45 U/kg/week. The sensitivity analyses are consistent with and provide robustness to the primary analysis conclusions.

Table 34. Sensitivity Analyses: Difference Between Epoetin Hospira and Epogen in Mean Weekly Hb and Mean Weekly Dose by Body Weight During the Last 4 Weeks of the Treatment Period in the Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)

Analysis Population	Difference Between Epoetin Hospira and Epogen in Mean Weekly Hemoglobin (g/dL) during Last 4 Weeks of the Treatment Period			Difference Between Epoetin Hospira and Epogen in Mean Weekly Epoetin Dose (U/kg/week) during Last 4 Weeks of the Treatment Period		
	Estimate of Difference LS Mean (SE)	90%CI*	95% CI**	Estimate of Difference LS Mean (SE)	90%CI*	95% CI**
Per Protocol EH: n = 204 EP: n = 192	-0.10 (0.083)	(-0.24, 0.03)	(-0.27, 0.06)	-2.41 (6.796)	(-13.61, 8.80)	(-15.77, 10.95)
Full Analysis Set EH: n = 161 EP: n = 153	-0.12 (0.085)	(-0.26, 0.02)	(-0.29, 0.04)	-6.48 (7.726)	(-19.22, 6.27)	(-21.68, 8.72)
Modified Full Analysis Set EH: n = 295 EP: n = 300	-0.13 (0.068)	(-0.24, -0.02)	(-0.26, 0.01)	-0.17 (5.597)	(-9.39, 9.05)	(-11.16, 10.82)
Retained Set EH: n = 301 EP: n = 305	-0.11 (0.068)	(-0.22, 0.01)	(-0.24, 0.03)	-5.37 (6.876)	(-16.70, 5.96)	(-18.87, 8.14)
ITT Excluding Closed Sites EH: n = 276 EP: n = 283†	-0.11 (0.069)	(-0.22, 0.01)	(-0.24, 0.03)	0.80 (5.538)	(-8.32, 9.92)	(-10.08, 11.68)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
†n was 283 for co-primary endpoint of Hb and 282 for co-primary endpoint of mean weekly dose
Abbreviations: CI = confidence interval; EH = subjects randomized to Epoetin Hospira; EP = subjects randomized to Epogen; LS = least square; n = number; SE = standard error
Note: LS Means and confidence intervals come from an ANCOVA model with fixed effect of Treatment and baseline as a covariate.

Additional sensitivity analyses were conducted using ~~a~~ multiple- imputation to explore the impact of missing data. In each study, the amount of missing data was similar between treatment groups. For EPOE-10-13, there was 9% (hemoglobin) and 10% (study drug dose) missing weekly data for each treatment group and for EPOE-10-01 there was 12% (hemoglobin) and 17% (study drug dose) missing weekly data for each treatment group. Twenty imputed datasets were generated using the Markov Chain Monte Carlo method assuming missing at random (MAR). Each imputed dataset was analyzed by the same ANCOVA model as in the primary analysis. The combined results across all the imputed datasets (Table 35) further demonstrate the robustness of the primary analysis.

Table 35. Sensitivity Findings from Multiple Imputation in the Subcutaneous and Intravenous Comparative Efficacy and Safety Studies

	Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)				Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)			
	Difference (Epoetin Hospira – Epogen)	SE	90% CI*	95% CI**	Difference (Epoetin Hospira – Epogen)	SE	90% CI*	95% CI**
Mean Weekly Hb (g/dL)	0.07	0.114	(-0.12, 0.26)	(-0.16, 0.29)	-0.20	0.08	(-0.33, -0.06)	(-0.36, -0.03)
Mean Weekly Dose (U/kg)	-0.48	6.56	(-11.32, 10.36)	(-13.42, 12.45)	-1.79	6.10	(-11.84, 8.26)	(-13.78,10.20)

*90% CI requested by FDA during 2017 BLA review
**95% CI pre-specified in Sponsor analysis as per protocol design submitted and agreed to by FDA during the development program
Abbreviations: SE = standard error

6.2.5.3. Subgroup Analyses on the Co-primary Endpoints

Subgroup analyses were conducted to determine any potential impact of intrinsic or extrinsic factors on results of the co-primary efficacy endpoints.

An evaluation of the effect of intrinsic parameters indicated that there were no clinically meaningful effects of sex, race, age, and body mass index (BMI) on the co-primary efficacy variables of mean weekly Hb and mean weekly dose by body weight between the treatment groups for both SC and IV administration (Table 36).

Table 36. Subgroup Analysis for the Co-Primary Endpoints in the Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13) and the Intravenous Comparative Efficacy and Safety Study (EPOE-10-01) (Intent-to-Treat Population)

			Variable during Last 4 Weeks of Treatment			
			N		90% CI for Difference	
Study	Factor	Level	Epoetin Hospira	Epogen	Mean Weekly Hb	Mean Weekly Dose/kg
Subcutaneous Comparative Efficacy and Safety Study (EPOE-10-13)	Sex	Female	60	66	(-0.22, 0.22)	(-10.04, 15.00)
		Male	64	56	(-0.19, 0.35)	(-24.35, 8.92)
	Race	Caucasian	69	59	(-0.22, 0.27)	(-11.19, 19.30)
		Black or African American	50	59	(-0.31, 0.19)	(-22.24, 6.13)
	Age	≤ 65	92	88	(-0.18, 0.22)	(-15.25, 7.46)
		>65 years	32	34	(-0.29, 0.39)	(-20.18, 26.14)
	BMI	<30 kg/m^2	65	62	(0.06, 0.56)	(-24.51, 4.14)
		≥ 30 kg/m^2	59	59	(-0.49, -0.02)	(-10.64, 18.85)
Intravenous Comparative Efficacy and Safety Study (EPOE-10-01)	Sex	Female	146	131*	(-0.18, 0.13)	(-17.21, 9.00)
		Male	160*	175	(-0.35, -0.05)	(-10.09, 15.27)
	Race	Caucasian	142	151	(-0.26, 0.05)	(-12.91, 15.77)
		Black or African American	149*	127*	(-0.31, 0.01)	(-5.98, 19.15)
	Age	≤ 65	231*	222*	(-0.24, 0.02)	(-6.01, 16.42)
		>65 years	75	84	(-0.44, -0.03)	(-25.26, 3.90)
	BMI	<30 kg/m^2	158	163	(-0.31, 0.00)	(-18.58, 8.45)
		≥ 30 kg/m^2	147	142	(-0.26, 0.03)	(-3.73, 20.09)

*The N for mean weekly dose/kg variable contains 1 less subject for this subgroup

Likewise, an evaluation of the effect of extrinsic parameters indicated that there was no clinically meaningful impact of etiology of renal disease, dose frequency at baseline, hypertension at baseline, diabetes at baseline, iron supplementation at baseline, and iron supplementation during treatment on the co-primary efficacy variables of mean weekly Hb and mean weekly dose by body weight between the treatment groups for both SC and IV administration.

6.2.5.4. Secondary Endpoints

For each week during the 16-week Maintenance Period of Study EPOE-10-13 (SC) and 24-week Treatment Period of Study EPOE-10-01 (IV), mean weekly Hb (Figure 40) and mean epoetin dose by body weight (Figure 41) were similar between the Epoetin Hospira and Epogen treatment groups.

A high and similar proportion of subjects in the Epoetin Hospira and Epogen groups had weekly mean Hb between 9 and 11 g/dL at week 16 in Study EPOE-10-13 (SC) (79.8% and 74.0%, respectively) at week 24 in Study EPOE-10-01 (IV) (73.2% and 71.4%, respectively)

A minority of subjects (4% in each treatment group in Study EPOE-10-13 [SC]; 6% in each treatment group in Study EPOE-10-01 [IV]) received a blood transfusion at any time during study participation.

Figure 40. Mean Weekly Hemoglobin Level (g/dL) (± SD) during the Maintenance Period of the Subcutaneous Comparative Efficacy and Safety Study (Study 10-13) and Treatment Period of the Intravenous Comparative Efficacy and Safety Study (Study 10-01) (Intent-to-Treat Population)

EPOE-10-13 **EPOE-10-01**

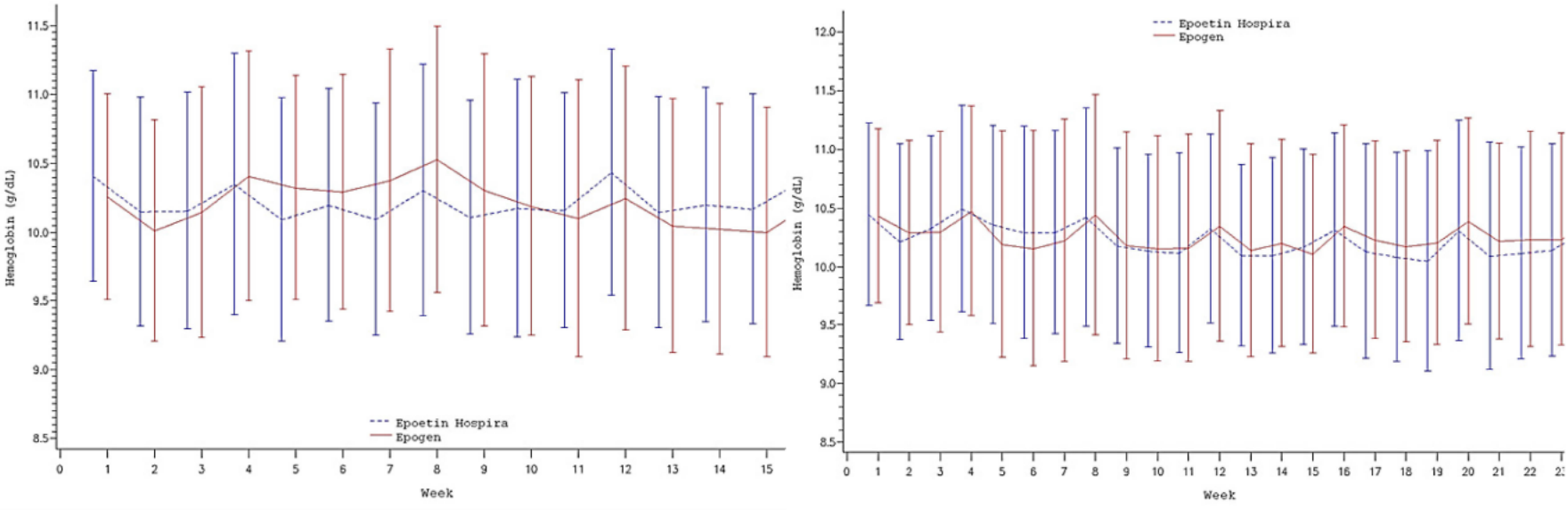
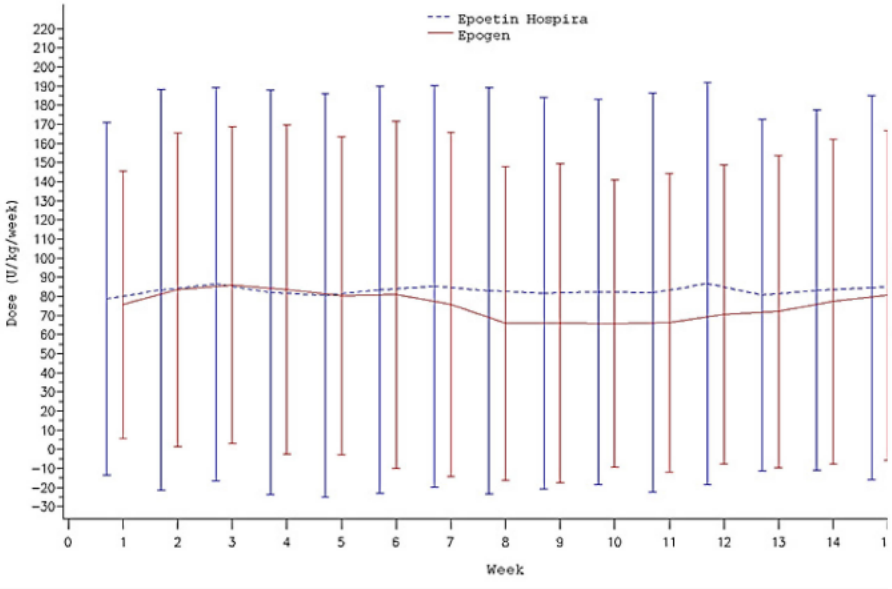
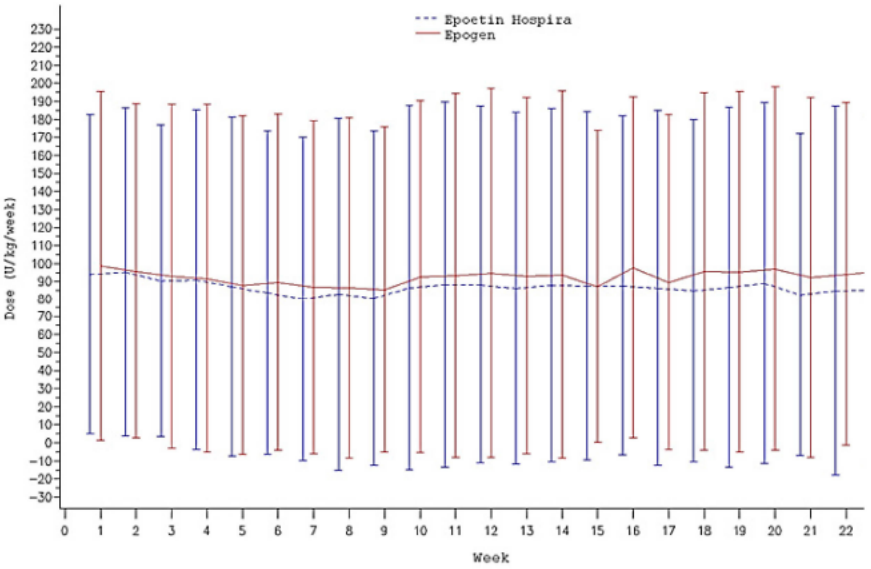


Figure 41. Mean Weekly Epoetin Dose by Body Weight (U/kg/week) (± SD) during the Maintenance Period of the Subcutaneous Comparative Efficacy and Safety Study (Study 10-13) and Treatment Period of the Intravenous Comparative Efficacy and Safety Study (Study 10-01) (Intent-to-Treat Population)

EPOE-10-13



EPOE-10-01



6.3. Clinical Safety

6.3.1. Summary of Safety

Safety analyses presented in this Briefing Document focus on the pooled experience in the two comparative safety and efficacy studies, EPOE-10-13 (SC) and EPOE-10-01 (IV).

Safety data for the two randomized controlled studies (treatment period in subjects with CKD on HD were pooled), allowing for a comparison with reference product control.

The safety profiles of Epoetin Hospira and Epogen reference product were comparable, supporting demonstration of biosimilarity of Epoetin Hospira to the reference product Epogen.

- The incidence of AEs, SAEs, and AEs leading to discontinuation were comparable between the randomized treatment groups.

- In the 2 randomized, controlled studies, there were 9 (2.1%) deaths in the Epoetin Hospira group and 9 (2.1%) deaths in the Epogen group. The Investigators considered all deaths either not or probably not related to study drug.

- The combined randomized Epoetin Hospira group and the combined randomized Epogen treatment groups were comparable based on incidence of events of interest, including hypertension (6.6% and 4.9%, respectively), myocardial infarction (0.9% and 0.7%), cerebrovascular events (0.9% and 1.4%), seizures (0.2% and 0.2%), potential allergic reactions (2.4% and 1.4%), and thromboembolic events (7.8% and 6.1%). Events of interest observed in the combined clinical studies were comparable with the type and incidences of AEs described in the Epogen US Package Insert (Epogen PI, 2014). There were no reported events of PRCA in the clinical program.

A systematic, program-wide evaluation of immunogenicity using well-established, validated methods for the assessment of antibody formation (that binds or neutralizes the effect of epoetin) showed a consistent immunogenicity profile of Epoetin Hospira and Epogen.

Overall, the safety profile of Epoetin Hospira is in line with the published literature for similar products, does not introduce any new safety signals, and is consistent with Epogen.

6.3.2. Safety Program Overview

Safety analyses presented in this Briefing Document focus on the experience in EPOE-10-13 (SC), EPOE-10-01 (IV), EPOE-11-04 (SC), and EPOE-11-03 (IV). The long-term safety studies (EPOE-11-04 [SC] and EPOE-11-03 [IV]) provide additional safety data, with exposure for up to an additional 48 weeks following exposure in EPOE-10-13 (SC) and EPOE-10-01 (IV). This allows for exposure to Epoetin Hospira for up to 64 weeks in SC administration and up to 72 weeks for IV administration. Overall, 707 subjects were treated with at least one dose of Epoetin Hospira with a mean exposure of 44 weeks.

The analysis populations are:

- The population analyzed for safety consisted of all subjects who received at least one dose of study drug during the Maintenance Period in EPOE-10-13 (SC) or during the Treatment Period in EPOE-10-01 (IV) (N = 423 and 426 subjects, respectively).

- The Long-term (LT) Population, which includes all subjects who received at least one dose of study drug in either EPOE-11-04 (SC) or EPOE-11-03 (IV) (N = 576).

The study completion rates (~85% of subjects completed the core studies) and discontinuation rates, including reasons for discontinuation, were comparable between the Epoetin Hospira and Epogen treatment groups (Table 29). Study drug exposure (approximately 18 weeks) and mean weekly study drug dose (85.8 and 86.8 U/kg/week, respectively) were comparable between the randomized Epogen and randomized Epoetin Hospira groups. Among subjects treated in a LTSS, the mean duration of study drug exposure and overall mean weekly study drug dose by body weight were approximately 40 weeks and 82.7 U/kg/week, respectively.

6.3.3. Adverse Events

ESAs have been associated with particular AEs. In general, these AEs are mechanism-based and an extension of ESA pharmacology; as such, they are not molecule-specific, but applicable to all ESAs. The particular AEs that have been reported with ESAs are summarized in the Warnings and Precautions section of the Epogen US Package Insert (Epogen PI, 2014). In the Epoetin Hospira clinical development program, these AEs have been characterized as events of interest.

6.3.3.1. Randomized, Controlled Trials

6.3.3.1.1. Treatment-Emergent Adverse Events

In both combined randomized treatment groups, approximately 75% of subjects experienced at least one TEAE. The common TEAEs were similar between treatment groups (Table 37).

Table 37. Treatment-Emergent Adverse Events for Combined Randomized Treatment Groups Occurring in at least 5% in Either Treatment Group

System Organ Class Preferred Term[a]	Epoetin Hospira Randomized (N = 423) n (%)	Epogen Randomized (N = 426) n (%)
Subjects with ≥ 1 TEAE	321 (75.9%)	318 (74.6%)
Gastrointestinal Disorders		
Diarrhea	26 (6.1%)	33 (7.7%)
Nausea	40 (9.5%)	33 (7.7%)
Vomiting	32 (7.6%)	21 (4.9%)
Injury, Poisoning, and Procedural Complication		
Arteriovenous Fistula Site Complication	32 (7.6%)	30 (7.0%)
Fall	22 (5.2%)	16 (3.8%)
Musculoskeletal and Connective Tissue Disorder		
Muscle Spasm	31 (7.3%)	28 (6.6%)
Pain in extremity	17 (4.0%)	22 (5.2%)
Nervous System Disorders		
Dizziness	23 (5.4%)	25 (5.9%)
Headache	29 (6.9%)	19 (4.5%)
Respiratory, Thoracic and Mediastinal Disorders		
Cough	21 (5.0%)	25 (5.9%)
Dyspnea	25 (5.9%)	26 (6.1%)
Vascular Disorder		
Hypertension	24 (5.7%)	19 (4.5%)
Hypotension	15 (3.5%)	29 (6.8%)

a. All investigator AE terms were coded using MedDRA dictionary version 14.1.
Note: Subjects are counted once within each SOC for each PT and may have had more than one AE.

6.3.3.1.2. Deaths, Serious Adverse Events, and Adverse Events Leading to Discontinuation

For the combined randomized treatment groups, 9 subjects (2.1%) in the Epoetin Hospira group and 9 subjects (2.1%) in the Epogen group experienced a treatment-emergent SAE resulting in death.

All deaths were considered by the Investigators to be probably not related or not related to study drug. Mortality observed in this clinical development program is consistent with what would be expected in a CKD population on HD receiving epoetin. A listing of subject deaths is presented in Table 46 in the Clinical Appendix.

In the combined, randomized groups, 101 (23.9%) Epoetin Hospira-treated subjects and 116 (27.2%) Epogen-treated subjects experienced at least one SAE. The incidence of the

most common SAEs was comparable between the two combined randomized treatment groups (Table 38).

Table 38. Treatment-Emergent Serious Adverse Events with Incidence ≥ 1% in Any Treatment Group for Combined Randomized Treatment Groups

System Organ Class Preferred Term[a]	Epoetin Hospira Randomized (N = 423) n (%)	Epogen Randomized (N = 426) n (%)
Number of Subjects with ≥ 1 Serious Event	101 (23.9%)	116 (27.2%)
Cardiac Disorders		
Cardiac Failure Congestive	5 (1.2%)	5 (1.2%)
General Disorders and Administration Site Conditions		
Non-cardiac Chest Pain	4 (0.9%)	8 (1.9%)
Infections and Infestations		
Cellulitis	3 (0.7%)	6 (1.4%)
Osteomyelitis	5 (1.2%)	1 (0.2%)
Pneumonia	7 (1.7%)	10 (2.3%)
Metabolism and Nutrition Disorders		
Fluid Overload	1 (0.2%)	7 (1.6%)
Hyperkalemia	4 (0.9%)	6 (1.4%)
Respiratory, Thoracic, and Mediastinal Disorders		
Dyspnea	3 (0.7%)	8 (1.9%)

a All investigator AE terms were coded using MedDRA dictionary version 14.1.
Note: Subjects are counted once within each SOC for each PT and may have had more than one SAE.

The incidences of TEAEs leading to study drug discontinuation were comparable between the randomized Epoetin Hospira (3.1%) and randomized Epogen (3.5%) groups.

Events of interest were identified prospectively based on the safety information of the Epogen/Procrit reference product, as described in the Epogen US Package Insert (Epogen PI, 2014), and grouped by standard Medical Dictionary for Regulatory Activities (MedDRA) query (SMQ) and grouping of Preferred Terms (PTs) for a medical concept in the absence of an SMQ were also conducted.

Events of interest, including SAEs, observed in the combined clinical studies, were comparable with the type and incidences of AEs described in the Epogen US Package Insert (Epogen PI, 2014). A summary of the events of interest by category is provided in (Table 39).

Table 39. Incidence of Class-Specific Adverse Events by Category for Combined Randomized Treatment Groups

System Organ Class or Grouping Preferred Term or Group[a]	Epoetin Hospira Randomized (N = 423) n (%)	Epogen Randomized (N = 426) n (%)
Thromboembolic Events	33 (7.8%)	26 (6.1%)
Hypertension	28 (6.6%)	21 (4.9%)
Potential Allergic Reactions	10 (2.4%)	6 (1.4%)
Myocardial Infarction	4 (0.9%)	3 (0.7%)
Cerebrovascular Events	4 (0.9%)	6 (1.4%)
Seizures	1 (0.2%)	1 (0.2%)
Pure Red Cell Aplasia	0	0

Further information for Adverse Events of Special Interest (AESIs) that were ≥ 1% between arms is provided below.

Thromboembolic Events

Patients with CKD on HD are known to be susceptible to thromboembolic events, and such events have been reported in patients with CKD receiving Epogen (Epogen PI, 2014).

The incidences of thromboembolic events (7.8% and 6.1%, respectively) in the randomized Epoetin Hospira and Epogen groups were in line with what has been reported in the product labeling for the reference product. In the combined randomized studies, 35.5% and 41.3% of the Epoetin Hospira and Epogen groups respectively had a medical history of thromboembolism with 8.7% and 9.6% respectively having a medical history of vascular access thrombosis at baseline. Examination of reported events of thromboembolism including number of events, incidence, seriousness, severity and treatment-relatedness supports a consistent profile between Epoetin Hospira and Epogen (Table 40). A listing of subjects with reported events of thromboembolism can be found in Table 47 in the Clinical Appendix.

Table 40. Summary of Thromboembolic Events for the Combined Randomized Treatment Groups

Parameter	Epoetin Hospira Randomized (N = 423) [n (%)]	Epogen Randomized (N = 426) [n (%)]
Number of Thromboembolic Events	39	36
Subjects with ≥ 1 Thromboembolic Event	33 (7.8%)	26 (6.1%)
Subjects with Serious Thromboembolic Events	8 (1.9%)	14 (3.3%)
Subjects with Severe Thromboembolic Events	5 (1.2%)	10 (2.3%)
Subjects with Treatment-Related Thromboembolic Events	0	1 (0.2%)

Hypertension

Patients with CKD on HD are recognized to have a high risk for hypertension. Accordingly, "following initiation and stabilization of Epogen, approximately 25% of patients on dialysis required initiation of or increases in antihypertensive therapy. Hypertensive encephalopathy and seizures have been reported in patients with CKD receiving Epogen" (Epogen PI, 2014).

In the combined randomized studies, over 98% of subjects in each treatment group had a medical history of hypertension at baseline. Slightly greater than 65% of the study population required between 2-5 antihypertensive medications. Examination of hypertension including number of events, incidence, seriousness, severity and treatment-relatedness supports a consistent profile between Epoetin Hospira and Epogen (Table 41). A listing of subjects with reported events of hypertension can be found in Table 48 in the Clinical Appendix.

Table 41. Summary of Hypertension Events for the Combined Randomized Treatment Groups

Parameter	Epoetin Hospira Randomized (N = 423) [n (%)]	Epogen Randomized (N = 426) [n (%)]
Number of Hypertension Events	33	32
Subjects with ≥ 1 Hypertension Event	28 (6.6%)	21 (4.9%)
Subjects with Serious Hypertension Events	3 (0.7%)	4 (0.9%)
Subjects with Severe Hypertension Events	1 (0.2%)	0
Subjects with Treatment-Related Hypertension Events	0	0

Further examination of the reported events of hypertension was conducted in tandem with objective blood pressure results in the clinical studies. Central tendency and extreme values of systolic blood pressure and diastolic blood pressure were consistent between the treatment

groups (Figure 45 and Figure 46). Overall, a comprehensive evaluation of the events of hypertension, in tandem with objective blood pressure data, reveals a consistent profile between Epoetin Hospira and Epogen.

Potential Allergic Reactions

Potential allergic reactions were identified prospectively based on the safety information of the Epogen/Procrit reference product, as described in the Epogen US Package Insert (Epogen PI, 2014). Potential allergic reactions consistent with anaphylaxis or angioedema were evaluated as AEs of Special Interest. This analysis, while sensitive to detect potential allergic reactions, generally identified reported events that had an alternative etiology or pertinent medical history that excluded true hypersensitivity. An evaluation of reported events did not identify AEs of hypersensitivity consistent with an immune response to Epoetin Hospira or Epogen. Potential allergic reactions consistent with anaphylaxis or angioedema were evaluated as a class-specific AE, most determined to have an alternative etiology or pertinent medical history that excluded true hypersensitivity to erythropoietin. Most events reported in this category were associated with fluid overload associated with underlying renal disease. Additionally, examination of TEAEs related to cutaneous events did not reveal any remarkable pattern of events between the two treatments (Table 42).

A listing of subjects with reported events of potential allergic reactions, along with pertinent medical history and alternative explanations for the event can be found in Table 49 in the Clinical Appendix.

Table 42. Summary of Potential Allergic Reactions Events for the Combined Randomized Treatment Groups

Parameter	Epoetin Hospira Randomized (N = 423) [n (%)]	Epogen Randomized (N = 426) [n (%)]
Number of Potential Allergic Reaction Events	11	6
Subjects with ≥ 1 Potential Allergic Reaction Event	10 (2.4%)	6 (1.4%)
Subjects with Serious Potential Allergic Reaction Events	0	1 (0.2%)*
Subjects with Severe Potential Allergic Reaction Events	0	1 (0.2%)*
Subjects with Treatment-Related Potential Allergic Reaction Events	0	0
*One subject (subject 13041-0038) in Study EPOE-10-01 who had been randomized to receive Epogen, experienced an AE of angioedema which was considered both serious and severe, and was attributed to the initiation of therapy with the angiotensin converting enzyme enalapril two days prior to the onset of the AE.		

6.3.3.2. Long-term Safety Studies of Epoetin Hospira

The LTSS EPOE-11-04 and EPOE-11-03 were open-label studies in subjects who completed the end-of-treatment assessments of their core studies, EPOE-10-13 and EPOE-10-01, respectively. Subjects in the LTSS received up to 48 additional weeks of Epoetin Hospira at the same regimen they had received study drug in the core studies in order to evaluate the

long-term safety of both SC and IV administration of Epoetin Hospira, including TEAEs and immunogenicity, and to provide supportive information regarding long-term efficacy.

Exposure

For the Safety Population, in the combined 48-week open-label LTSS, EPOE-11-04 and EPOE-11-03, the mean duration of study drug exposure and overall mean weekly study drug dose by body weight were approximately 40 weeks, and 82.7 U/kg/week, respectively, which is comparable to the exposure in the randomized studies.

Treatment-Emergent Adverse Events

In the combined 48-week open-label LTSSs, 86.5% of subjects experienced at least one TEAE. The common TEAEs (incidence ≥ 5%) were anemia, arteriovenous fistula site complication, back pain, cough, diarrhea, dizziness, dyspnea, headache, hypotension, hyperkalemia, hypertension, muscle spasms, nausea, pain in extremity, peripheral edema, pneumonia, pyrexia, upper respiratory tract infection, and vomiting.

Deaths, Serious Adverse Events, and Adverse Events Leading to Discontinuation

For the combined LTSSs, 43 subjects (7.5%) experienced a treatment-emergent SAE resulting in death; 40 of the TEAEs resulting in death were considered by the Investigators to be probably not related or not related to study drug. Three subjects had TEAEs resulting in death considered by the Investigators to be possibly related to study drug: intracerebral hemorrhage, myocardial infarction, and cardio-respiratory arrest.

In the combined LTSS, 39.4% of subjects reported at least one SAE, the five most common being pneumonia (3.6%), sepsis (3.3%), congestive cardiac failure (2.8%), hyperkalemia (2.6%), and acute myocardial infarction (2.3%).

In the combined LTSS, 6.6% of subjects experienced an AE leading to study drug discontinuation, the most common being cardiac arrest (4 subjects, 0.7%), congestive heart failure (4 subjects, 0.7%), cardio-respiratory arrest (3 subjects, 0.5%), cerebral hemorrhage (3 subjects, 0.5%), acute myocardial infarction (2 subjects, 0.3%), myocardial infarction (2 subjects, 0.3%), nausea (2 subjects, 0.3%), sepsis (2 subjects, 0.3%), and septic shock (2 subjects, 0.3%).

In the LTSS, there were no new safety signals identified. The LTSS provide additional data that the profile of Epoetin Hospira is consistent with what has been historically seen with the reference product, Epogen.

6.3.4. Clinical Laboratory, Vital Signs, and Electrocardiogram Findings

Laboratory, vital signs, or electrocardiogram (ECG) assessments were comparable between the Epoetin Hospira and Epogen groups.

6.3.5. Immunogenicity

6.3.5.1. Summary of Immunogenicity

A systematic, program-wide evaluation of immunogenicity was performed using well-established, validated methods for the assessment of antibody formation.

- No neutralizing antibodies against rhEPO were detected in any subject.
- There were no reported events of Pure Red Cell Aplasia (PRCA) in the clinical program.
- None of the reported events of potential allergic reactions were medically determined to be hypersensitivity reactions potentially consistent with an immune response to epoetin.

The immunogenicity profiles of Epoetin Hospira and Epogen were comparable.

6.3.5.2. Immunogenicity Results from Clinical Studies

In the Epoetin Hospira development program, a systematic, program-wide evaluation of clinical immunogenicity was performed in accordance with *FDA Guidelines for Immunogenicity Testing* (FDA 2009; FDA 2014a). Serum samples were taken pre-dose prior to first dose of study drug, at intervals throughout the study, at the end of the treatment periods, and at the follow-up period, if applicable. Per FDA recommendation, the radioimmunoprecipitation (RIP) assay and the neutralizing anti-recombinant human erythropoietin (anti-rhEPO) assay were updated with more stringent validated cut points and these cut points were employed in the immunogenicity data analyses (Table 43).

Across the Epoetin Hospira development program, immunogenicity did not impact the conclusions drawn from the PK, PD, efficacy, and safety data. Table 43 provides a summary of the comparative immunogenicity results across the randomized studies for subjects who were ADA positive at baseline or at any time during the treatment period. The incidence of ADA-positive subjects at any time during the treatment period was consistent between Epoetin Hospira (4 subjects [1.0%]) and Epogen (4 subjects [1.0%]. Table 44 lists subjects in the combined randomized studies who were ADA positive at baseline or at any time during the treatment period. The incidence of treatment-emergent ADA-positive subjects, i.e., subjects who were not positive at baseline and became positive during the treatment period in the combined randomized studies was consistent between Epoetin Hospira (2 subjects [0.5%]) and Epogen (3 subjects [0.7%]). Table 50 in the Clinical Appendix provides a listing of the 12 subjects in the combined randomized studies who were ADA positive. The majority of these subjects had binding antibodies with titers of ≤ 1:2 as measured by the more stringent RIP assay cut points. In the LTSS, 9 subjects were ADA positive at baseline or at some time during the treatment period, and there was no impact of immunogenicity on the observed efficacy or safety of Epoetin Hospira. Across the entire clinical program, neutralizing antibodies against recombinant human epoetin (rhEPO) were not detected in any subject.

Additional evaluation of the potential immunogenicity risk included an assessment of adverse events reported in the development program. There were no reported events of

PRCA in any subject in the clinical program. An evaluation of reported events did not identify AEs of hypersensitivity consistent with an immune response to Epoetin Hospira or Epogen. Potential allergic reactions consistent with anaphylaxis or angioedema were evaluated as a class-specific AE. Most of these reactions were determined to have an alternative etiology or pertinent medical history that excluded true hypersensitivity to erythropoietin (Table 49 in the Clinical Appendix). Additionally, examination of TEAEs related to cutaneous events did not reveal any remarkable pattern of events between the two treatments. Overall, the immunogenicity profile of Epoetin Hospira was comparable to that of Epogen.

Table 43. Immunogenicity Testing Results by Updated Assay for the Combined Randomized Treatment Groups

Visit Number of Subjects with Sample at Visit Assay Result	Epoetin Hospira Randomized (N = 423)	Epogen Randomized (N = 426)
Baseline		
Number of subjects with sample at visit	378	370
Negative RIP[a] [n (%)]	375 (99.2)	366 (98.9)
Positive RIP[b] [n (%)]	3 (0.8)	4 (1.1)
Positive Neutralizing Antibody[c] [n (%)]	0	0
At Any Time During Treatment Period		
Number of subjects with sample at visit	393	397
Negative RIP[a] [n (%)]	389 (99.0)	393 (99.0)
Positive RIP[b] [n (%)]	4 (1.0)	4 (1.0)
Positive Neutralizing Antibody[c] [n (%)]	0	0

Abbreviations: N = number of subjects in the Safety Population for each treatment group; n = number of subjects with a positive or negative result for the assay; RIP = radioimmunoprecipitation

Note: Baseline includes all samples taken prior to first exposure of study drug for each study.

Note: During Treatment Period includes all samples taken between first exposure and last exposure of study drug

a. Negative screening radioimmunoprecipitation assay result or negative confirmatory radioimmunoprecipitation assay result.

b. Positive confirmatory radioimmunoprecipitation assay result.

c. Positive neutralizing antibody based on assay in which cell proliferation is dependent on epoetin, and presence of neutralizing antibody decreases cell proliferation; assay only performed on samples with a positive confirmatory RIP assay result.

Table 44. Listing of Subjects with Positive ADA Result Measured by Updated RIP Assay by Time Period (Combined Randomized Treatment Groups, Safety Population)

Visit	Epoetin Hospira Randomized	Epogen Randomized
Baseline	11095-0478	
	14054-0310	
	21012-0109	
		11045-0276
		13028-0260
		14023-0350
		24020-0027
At Any Time During Treatment Period	11095-0478	
	14040-0560	
	14054-0310	
	23015-0057	
		11045-0276
		14071-0591
		21001-0132
		24005-0053

Abbreviations: ADA = anti-drug antibody; RIP = radioimmunoprecipitation
Note: Baseline includes all samples taken prior to first exposure of study drug for each study.
Note: During Treatment Period includes all samples taken between the first exposure and the last exposure of study drug.

7. EXTRAPOLATION OF EVIDENCE FOR BIOSIMILAR TO ALL EPOGEN/PROCRIT REFERENCE PRODUCT INDICATIONS

7.1. Summary of Extrapolation

Under the abbreviated biosimilar pathway, the evaluation of biosimilarity is based on the totality of evidence obtained from analytical, nonclinical and clinical studies. For Epoetin Hospira, the totality of evidence supports a demonstration of biosimilarity to the Epogen/Procrit reference product, including comparative clinical data in CKD on HD. Additional indications for the reference product include treatment of anemia in adult patients with CKD not on dialysis; treatment of anemia in zidovudine-treated HIV-infected adult patients; and treatment of anemia in myelosuppressive chemotherapy-treated adult patients, as well as other conditions of use. Therefore, information regarding the safety, purity, and potency of Epogen/Procrit in its additional licensed conditions of use may be extrapolated to the proposed biosimilar product based on a robust scientific justification. Specific considerations and how they are addressed are provided below.

- Mechanism of action in each condition of use:
    - Relative or absolute erythropoietin deficiency contributes to anemia in all approved indications for Epogen.
    - The mechanism of action to stimulate erythropoiesis is common to all indications for Epogen/Procrit reference product.
    - Comparative analytical biosimilarity functional assay results support same mechanism of action of Epoetin Hospira and Epogen/Procrit reference product.
- Pharmacokinetics and Pharmacodynamics:
    - There is a well characterized PK/PD relationship that generalizes across multiple epoetin products in healthy subjects and across all patient populations for which Epogen/Procrit reference product is indicated.
    - PK/PD equivalence was established between Epoetin Hospira and Epogen under single-dose and multiple-dose conditions.
- Expected toxicities, including immunogenicity:
    - Safety evaluation was conducted in CKD, which is the most sensitive model, as historical risk of PRCA is greatest in this population that also tends to be less immunocompromised than other conditions such as chemotherapy-induced anemia (CIA).
    - There is a well-characterized safety profile of Epogen/Procrit reference product across indications primarily driven by PD response that was equivalent between

Epoetin Hospira and Epogen in comparative single-dose and multiple-dose PK/PD studies.

- Similar comparative safety of Epoetin Hospira and Epogen reference product was observed in two sensitive populations: CKD on HD under SC and IV conditions and in healthy subjects under SC conditions.

- Any other factor that may affect safety or efficacy:
  - Route of administration: Both routes of administration (SC and IV) were tested in a sensitive clinical model with equivalence in efficacy demonstrated and comparable safety observed between Epoetin Hospira and the Epogen reference product to support extrapolation to all clinical conditions approved for the reference product.
  - Formulation: Clinically inactive ingredients in Epoetin Hospira and Epogen reference product do not impact PK/PD similarity and subsequently, safety or efficacy.

- The establishment of PD similarity in healthy subjects under single and multiple dose conditions provides direct clinical evidence of equivalence in this non-anemic target population. The healthy subject population is representative of the population for whom the product is indicated for reduction of allogeneic RBC transfusions in patients undergoing elective, noncardiac, nonvascular surgery.

The totality of evidence along with the scientific justification data support extrapolation to all other indications currently approved for the Epogen/Procrit reference product.

7.2. Rationale for Other Indications and Dose Regimens

Per the *FDA Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product* (FDA 2015a), scientific justification for extrapolation should address the following issues for the tested and extrapolated conditions of use:

- the mechanism(s) of action in each condition of use for which licensure is sought; this may include:
  - the target/receptor(s) for each relevant activity/function of the product;
  - the binding, dose/concentration response and pattern of molecular signaling upon engagement of target/receptors;
  - the relationships between product structure and target/receptor interactions;
  - the location and expression of the target/receptor(s);
- the PK and bio-distribution of the product in different patient populations (relevant PD measures also may provide important information on the mechanism of action);

- the immunogenicity of the product in different patient populations
- differences in expected toxicities in each condition of use and patient population (including whether expected toxicities are related to the pharmacological activity of the product or to "off-target" activities); and
- any other factor that may affect the safety or efficacy of the product in each condition of use and patient population for which licensure is sought.

Evidence supporting extrapolation based on each of these points is discussed below.

7.2.1. Ubiquity of the Mechanism of Action

Both endogenous erythropoietin and rhEPO can interact with the homodimeric erythropoietin receptor (EPO-R) on particular cells in the erythropoietic lineage, including mature burst-forming unit-erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), pro-erythroblast, and basophilic erythroblast, to initiate the same activation pathways, and lead to erythropoiesis by preventing apoptosis of the cells as they mature (Koury and Bondurant, 1992). The interaction of rhEPO with its receptor on these cells, and its prevention of apoptosis of these cells are independent of the cause of anemia in anemic patients. The same mechanism also underlies the drug effect in patients receiving rhEPO to reduce the need for transfusion perioperatively. The central therapeutic effect across all indications and conditions of use is epoetin stimulates erythropoiesis through the same mechanism as endogenous erythropoietin (Jelkmann, 2007).

7.2.2. Pharmacokinetics/Pharmacodynamics

PK/PD Similarity of Epoetin Hospira and Epogen following Single-Dose Administration

Study EPOE-12-02 compared the PK and PD of epoetin following the SC administration of a single-dose of 100 U/kg of Epoetin Hospira and Epogen to healthy male subjects. The results demonstrate PK and PD equivalence in support of PK/PD similarity of Epoetin Hospira and Epogen under single-dose conditions.

PK/PD Similarity of Epoetin Hospira and Epogen following Multiple-Dose Administration

Study EPOE-14-01 compared the PK and PD of epoetin following the SC administration over 26 days of 12 fixed doses of 100 U/kg each of Epoetin Hospira or Epogen to healthy male subjects. The results demonstrated the PK and PD equivalence in support of similarity of Epoetin Hospira and Epogen under multiple-dose conditions.

PK of Epogen/Procrit

Several lines of evidence indicate that the PK profile of Epogen/Procrit is consistent across populations, including healthy adults, adult patients with various disease conditions, and pediatric patients. The 2014 Epogen Package Insert includes a PK summary that states that in adult and pediatric patients with CKD, the elimination half-life ($t_{½}$) of plasma epoetin after

IV administration of Epogen ranged from 4 to 13 hours. After SC administration, C_{max} was achieved within 5 to 24 hours. The $t_{½}$ in adult patients with serum creatinine greater than 3 mg/dL was similar between those not on dialysis and those maintained on dialysis. The PK data indicate no apparent difference in Epogen $t_{½}$ among adult patients above or below 65 years of age, and further indicate that the PK profile of Epogen in children and adolescents appears similar to that of adults. After three weeks of SC Epogen administered to anemic cancer patients and healthy controls, the concentration-time profiles were similar (FDA Clinical Pharmacology Review, 2004). In addition, Elliott et al. (2008) reported that the PK characteristics of epoetin in several other populations, including patients with CKD, liver cirrhosis, and myelodysplastic syndrome, appear similar or comparable to those in healthy subjects.

PK/PD of Epogen/Procrit

Time during which Epogen/Procrit concentrations are above a minimum effective concentration is the main determinant of efficacy in increasing Hb levels (Doshi et al., 2013). In this regard, the recommended dose of the reference product Epogen/Procrit for each approved indication varies with the indication, and is generally based initially on weight. For anemia, individualized dose adjustments are necessary to maintain Hb levels in the target range in individual patients across all indications (Epogen PI, 2014). In patients undergoing elective, noncardiac, nonvascular surgery, the recommended dosing regimens are fixed, and administered prior to and on the day of surgery.

7.2.3. No Toxicity Differences Among Conditions of Use

The reference product Epogen/Procrit has been used to safely treat patients with various etiologies of anemia since 1989. The safety profile of Epoetin Hospira is comparable to the reference product as assessed in the clinical development program for Epoetin Hospira.

7.2.4. Similarity in Efficacy and Safety between Epoetin Hospira and Epogen

The clinical program supports a determination that Epoetin Hospira is highly similar to Epogen/Procrit reference product. PK/PD equivalence was demonstrated in the most discerning clinical model under single and multiple fixed-dose conditions in healthy subjects. Additionally, equivalence was established in two well-controlled comparative efficacy and safety studies in renal anemia with SC and IV administration. The safety profile, including the immunogenicity profile, is consistent and comparable between Epoetin Hospira and Epogen reference product, with no clinically meaningful differences.

Therefore, information regarding the safety, purity, and potency of Epogen/Procrit in its additional licensed conditions of use may be extrapolated to Epoetin Hospira.

7.3. Risk Evaluation and Mitigation Strategy

FDA recently communicated in April 2017 a change in requirements for REMS for erythropoiesis-stimulating agents (ESAs). Specifically, FDA determined that the ESA Risk Evaluation and Mitigation Strategy (REMS), which was limited to the use of Epogen/Procrit and Aranesp to treat patients with anemia due to associated myelosuppressive chemotherapy,

is no longer necessary to ensure that the benefits of Epogen/Procrit and Aranesp outweigh its risks of shortened overall survival and/or increased risk of tumor progression or recurrence in patients with cancer. Pfizer is committed to working with FDA to ensure robust pharmacovigilance measures for Epoetin Hospira aligned with current FDA expectations and consistent with the Epogen/Procrit reference product and ESA class.

8. POSTMARKETING SURVEILLANCE

Epoetin Hospira Injection (hereafter Epoetin Hospira) is related to and originated from the development of Hospira's EU biosimilar, Retacrit™. EU-approved Retacrit is a human recombinant epoetin biosimilar to Eprex® (EU approved epoetin alfa), with indications for treatment of anemia associated with chronic renal failure or chemotherapy for solid tumors, malignant lymphoma, or multiple myeloma. EU-approved Retacrit was approved in compliance with the European Medicines Agency (EMA) guidelines for the development of biosimilar recombinant erythropoietin and meets the European Pharmacopoeia monograph requirements for erythropoietin. In accordance with these guidelines, biosimilarity of EU-approved Retacrit to the Eprex reference product has been established. The information included in this section regarding EU-approved Retacrit is not part of the data package for the biosimilarity assessment of Epoetin Hospira to the Epogen/Procrit reference product. As discussed with FDA, EU-Approved Retacrit post-marketing safety data is provided as supportive information only for a related product.

The International Birth Date (IBD) of the EU-Approved Retacrit is 18 December 2007, based on first approval in the EU through the Centralized Procedure. It is currently licensed in over 30 countries worldwide.

The estimated cumulative post-marketing exposure for Hospira EU-approved Retacrit, from 18 December 2007 to 01 May 2016 using the World Health Organization Defined Daily Dose, was approximately 323,108 patient-years.

During this period of exposure, there have been two reported cases of PRCA possibly related to EU-approved Retacrit. The first case was confirmed by positive neutralizing anti-erythropoietin antibody results and bone marrow biopsy and was reported from the ongoing post-authorization safety study (PASCO II). The second case was a spontaneously reported case confirmed by positive anti-erythropoietin antibody and weakly positive neutralizing anti-erythropoietin antibodies with a bone marrow examination consistent with a definitive diagnosis of PRCA. While the causality assessment in the second case was considered related to EU-Retacrit treatment, the case was confounded by prior treatment with another ESA (Mircera: methoxy polyethylene glycol-epoetin beta) during which the patient experienced lack of effect and subsequently was switched to treatment with Retacrit. Upon review of these suspected cases of PRCA for EU-approved Retacrit, the potential for immunogenicity appears consistent with the known safety profile for the EU-approved reference product.

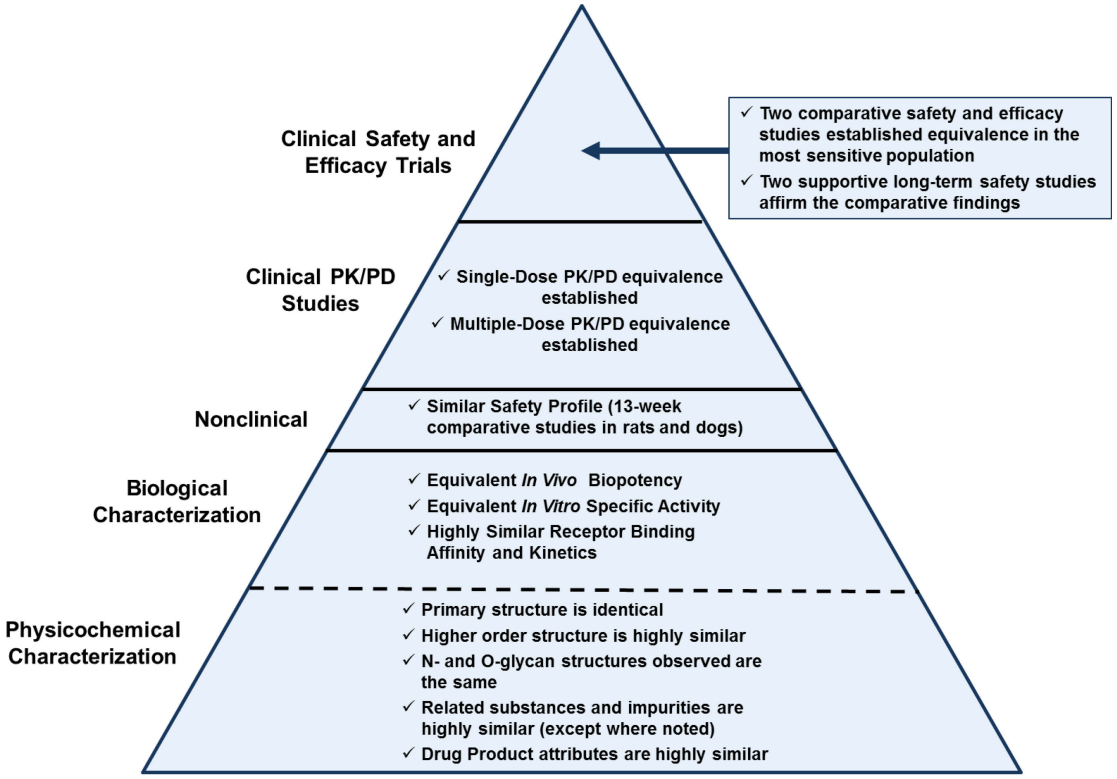
Overall, the severity and nature of AESIs is similar to what has been known for the EU-approved reference product. The analysis of post-marketing AESI reports did not suggest any meaningful differences between EU-approved Retacrit and the Eprex reference product. Thus, the safety profile of the EU-approved Retacrit, based on review of reported AESI both in post-marketing studies as well as from spontaneous reporting, is consistent with the EU approved reference product as well as the safety profile of US-approved Epogen/Procrit (Epogen PI, 2014).

The overall safety observations remain consistent with the safety profile described for the EU approved reference product and the benefit-risk profile for EU-approved Retacrit remains favorable when used in accordance with current product information.

9. CONCLUSIONS

The comprehensive Epoetin Hospira development program supports the approval of Epoetin Hospira as a biosimilar to the Epogen/Procrit reference product meeting the statutory definition of "biosimilar" as stated in Section 351(k) of the PHS Act. The totality of evidence in the Epoetin Hospira program (Figure 42) across the comparative foundational analytical assessment, nonclinical data, clinical PK/PD studies, and the comparative clinical efficacy and safety studies provides the necessary data to determine that the statutory pillars of biosimilarity have been satisfied.

Figure 42. Totality of Evidence from the Epoetin Hospira Development Program



The extensive comparative structural and functional characterization for Epoetin Hospira and the Epogen/Procrit US-licensed reference product completed as part of the Epoetin Hospira development program provides the foundation for the biosimilarity assessment. Epoetin Hospira has an identical primary structure and highly similar higher order structure to Epogen/Procrit.

Across the comparative epoetin analytical attributes, *in vitro* specific activity is most indicative of the inherent activity of the epoetin protein and *in vivo* biopotency is most indicative of *in vivo* performance. These two functional attributes are most important in

the analytical assessment of biosimilarity. Equivalence of the *in vitro* Specific Activity and *in vivo* biopotency attributes between Epoetin Hospira and the Epogen/Procrit reference product was demonstrated using formal equivalence testing with pre-specified criteria.

The demonstration of functional equivalence was further corroborated in the discerning comparative single-dose and multiple-dose PK/PD studies performed in healthy subjects that demonstrate no *in vivo* performance differences between Epoetin Hospira and Epogen.

In addition, the most pertinent clinical data under conditions of SC or IV therapeutic use in subjects with anemia secondary to CKD on HD, a sensitive population for which the reference product is indicated, demonstrated no clinical meaningful differences in efficacy or safety between Epoetin Hospira and the Epogen reference product (EPOE-10-13 [SC] and EPOE-10-01 [IV]).

Collectively, the comparative clinical data further support the conclusions of the foundational analytical assessment and demonstrate that Epoetin Hospira is highly similar to the Epogen/Procrit reference product notwithstanding minor differences in clinically inactive components.

Establishment of biosimilarity from the totality of evidence in subjects with CKD on HD as well as in healthy volunteers following multiple dose administration, a sensitive model in the overall immunogenicity assessment conducted across the Epoetin Hospira clinical program, enable extrapolation of the safety and efficacy data to all other indicated conditions of use.

In summary, Epoetin Hospira met all of the regulatory requirements for biosimilarity. The totality of data demonstrates that Epoetin Hospira is highly similar to the Epogen/Procrit reference product with no clinically meaningful differences in terms of the safety, including immunogenicity, purity, and potency.

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11. APPENDICES

11.1. Analytical Appendices

Epoetin Content Target Change

As noted in *Section 3.2, Drug Product Overview*, a minor revision to the Epoetin Hospira DP content target, representing a shift in the epoetin content target of approximately 3.5%, was implemented during the BLA review. The epoetin content target change was implemented in consultation with FDA to enhance the similarity of the Epoetin Hospira Drug Product (DP) to the Epogen/Procrit reference product. This small epoetin content difference can be distinguished using the highly sensitive Epoetin Content RP-HPLC analytical method but does not impact functional activity measured using the *In Vivo* Biopotency and *In Vitro* Biopotency assays as described below. In addition, the results from the clinical PK/PD and comparative safety and efficacy studies demonstrate that the minor differences in epoetin content between the Epoetin Hospira lots and the Epogen/Procrit reference product are not clinically meaningful.

The revised epoetin content target was established as the mean epoetin content for the Epogen/Procrit reference product lots. The revised target was used to manufacture nine lots of Epoetin Hospira DP in July 2015. This target will be also be used to manufacture all future commercial lots of the Epoetin Hospira DP. Establishment of the revised target as the mean measured epoetin content for the Epogen/Procrit reference product, coupled with the establishment of product specifications consistent with the range of measured epoetin content results for the Epogen/Procrit reference product, ensures control of the Epoetin Hospira DP within the measured range of the reference product.

The mean epoetin content for the nine Epoetin Hospira DP lots manufactured using the revised target was shifted slightly above the revised epoetin content target (+1.8%). This small shift is due to normal process variability. Over time, with additional manufacturing, the epoetin content results for commercial Epoetin Hospira lots are expected to be normally distributed around the revised epoetin content target.

A comparison of the *In Vivo* Biopotency results for the Epoetin Hospira lots manufactured using the original and revised epoetin content targets and the epoetin content results for the Epogen/Procrit reference product are shown in Figure 43. The minor differences in epoetin content between the lots manufactured at the original and revised targets and the Epogen/Procrit reference product do not lead to differences in the measured biopotency determined using the *in vivo* mouse bioassay. The Epoetin Hospira lots manufactured using both the original and revised epoetin content targets have *In Vivo* Biopotency results completely within the range of the Epogen/Procrit reference product results. The *In Vitro* Biopotency results for the Epoetin Hospira lots manufactured using the original and revised targets and the Epogen/Procrit reference product, shown in Figure 44, are consistent with the *In Vivo* Biopotency results. The *In Vitro* Biopotency results show no differences between the Epoetin Hospira lots manufactured at the original and revised targets and the Epogen/Procrit reference product.

The *In Vivo* Biopotency and *In Vitro* Biopotency functional assay results demonstrate that the small epoetin content differences between the Epoetin Hospira lots manufactured using the original and revised targets and the Epogen/Procrit reference product are not biologically meaningful.

Figure 43. Epoetin Content and *In Vivo* Biopotency Results for Epoetin Hospira Lots (Original and Revised Content Target) and Epogen/Procrit Reference Product Lots

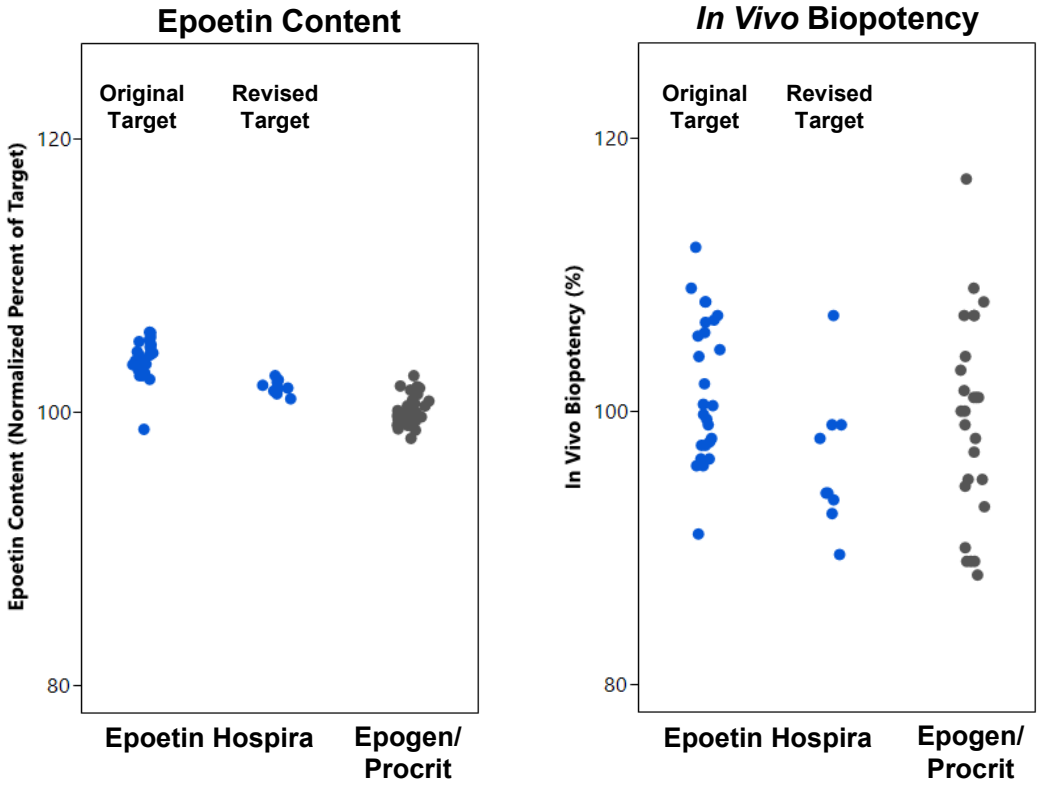
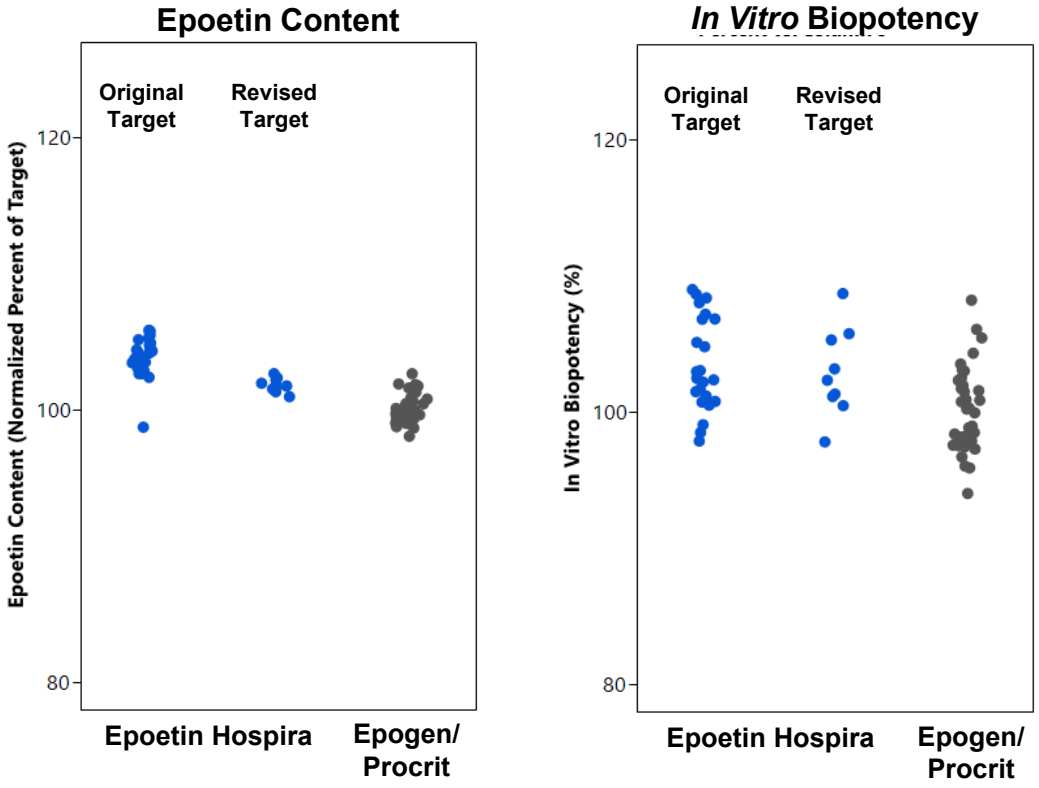


Figure 44. Epoetin Content and *In Vitro* Biopotency Results for Epoetin Hospira Lots (Original and Revised Content Target) and Epogen/Procrit Reference Product Lots



11.2. Clinical Appendices

Table 45. Serious Adverse Events Resulting in Death for the Combined Randomized Studies (Enrolled Population)

System Organ Class Preferred Term[a]	Epoetin Hospira Randomized (N = 423) n (%)	Epogen Randomized (N = 426) n (%)
Subjects with SAE resulting in death	**9 (2.1%)**	**9 (2.1%)**
Cardiac Disorders	**1 (0.2%)**	**7 (1.6%)**
Angina Pectoris	0	1 (0.2%)
Arrhythmia	0	1 (0.2%)
Cardiac Arrest	1 (0.2%)	3 (0.7%)
Cardio-respiratory Arrest	0	2 (0.5%)
General Disorders and Administration Site Conditions	**1 (0.2%)**	**0**
Sudden Death	1 (0.2%)	0
Gastrointestinal Disorders	**1 (0.2%)**	**0**
Gastrointestinal hemorrhage	1 (0.2%)	0
Infections and Infestations	**2 (0.5%)**	**0**
Infectious Peritonitis	1 (0.2%)	0
Sepsis	1 (0.2%)	0
Nervous System Disorders	**1 (0.2%)**	**0**
Metabolic Encephalopathy	1 (0.2%)	0
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	**0**	**1 (0.2%)**
Lung Cancer Metastatic	0	1 (0.2%)
Renal and Urinary Disorders	**3 (0.7%)**	**0**
Azotemia	3 (0.7%)	0
Vascular Disorders	**0**	**1 (0.2%)**
Aortic Stenosis	0	1 (0.2%)

a. All SAE System Organ Class and Preferred Terms were coded using MedDRA dictionary version 14.1.

Table 46. Listing of Serious Adverse Events Resulting in Death for the Combined Randomized Studies (Enrolled Population)

For the Combined Randomized Studies, during the Maintenance Period of Study EPOE-10-13 and the Treatment Period of Study EPOE-10-01, SAEs resulting in death occurred in 9 subjects (2.1%) in the Epoetin Hospira treatment group and 9 subjects (2.1%) in the Epogen treatment group.

Study Period Treatment	Subject ID Age/Race/Sex	Treatment -Emergent (Yes/No)	S: System Organ Class P: Preferred Term V: Verbatim Term	S: Start Date (Rel. Day) E: Stop Date (Rel. Day)	O: Outcome S: Severity A: Action Taken with Study Drug X: Other Action Taken	R: Relationship[a] A: Alternative Etiology Y: Seriousness Criteria[b]
EPOE-10-13 Maintenance Epoetin Hospira	24011-0189 54/W/M	Yes	S: Gastrointestinal Disorders P: Gastrointestinal Haemorrhage V: Gastrointestinal Bleed	S: (b) (6) (67) E: (b) (6) (68)	O: Fatal S: Severe A: Drug Withdrawn X: Treatment Required	R: Not Related A: Underlying Illness Y: 1,4
EPOE-10-13 Maintenance Epoetin Hospira	25013-0317 67/W/F	Yes	S: Cardiac Disorders P: Cardiac Arrest V: Death Due To Cardiac Arrest	S: (b) (6) (73) E: (b) (6) (73)	O: Fatal S: Severe A: Not Applicable X: None	R: Not Related A: Cardiac Arrest Cause Unknown Y: 1
EPOE-10-13 Maintenance Epoetin Hospira	21001-0277 77/W/F	Yes	S: Renal And Urinary Disorders P: Azotaemia V: Uremia	S: (b) (6) (54) E: (b) (6) (54)	O: Fatal S: Severe A: Not Applicable X: None	R: Not Related A: Withdrawl From Routine Dialysis Y: 1
EPOE-10-13 Maintenance Epogen	24017-0077 65/W/M	Yes	S: Cardiac Disorders P: Arrhythmia V: Sudden Death Due To Cardiac Arrythymia Secondary To Atherosclerotic Cardiovascular Disease	S: (b) (6) (39) E: (b) (6) (39)	O: Fatal S: Severe A: Dose Not Changed X: None	R: Probably Not Related A: History Diabetes, Coronary Artery Disease Y: 1
EPOE-10-13 Maintenance Epogen	25003-0307 64/B/M	Yes	S: Vascular Disorders P: Aortic Stenosis V: Acute Aortic Stenosis	S: (b) (6) (78) E: (b) (6) (87)	O: Fatal S: Severe A: Not Applicable X: Treatment Required	R: Probably Not Related A: Aortic Stenosis Y: 1,4,6

Study Period Treatment	Subject ID Age/Race/Sex	Treatment -Emergent (Yes/No)	S: System Organ Class P: Preferred Term V: Verbatim Term	S: Start Date (Rel. Day) E: Stop Date (Rel. Day)	O: Outcome S: Severity A: Action Taken with Study Drug X: Other Action Taken	R: Relationship[a] A: Alternative Etiology Y: Seriousness Criteria[b]
EPOE-10-01 Treatment Epoetin Hospira	11003-0118 57/W/F	Yes	S: General Disorders and Administration Site Conditions P: Sudden Death V: Sudden Death Cause Unknown	S: (b) (6) (38) E: (b) (6) (38)	O: Fatal S: Severe A: Drug withdrawn X: None	R: Probably Not Related A: Unknown Y: 1
EPOE-10-01 Treatment Epoetin Hospira	11033-0086 62/W/F	Yes	S: Infections and Infestations P: Sepsis V: Sepsis	S: (b) (6) (139) E: (b) (6) (157)	O: Fatal S: Severe A: Drug withdrawn X: Other: hospitalization	R: Probably Not Related A: Pneumonia Y: 1,2,4
EPOE-10-01 Treatment Epoetin Hospira	11033-0156 51/W/M	Yes	S: Nervous System Disorders P: Metabolic Encephalopathy V: Toxic Metabolic Encephalopathy	S: (b) (6) (180) E: (b) (6) (193)	O: Fatal S: Severe A: Dose not changed X: Other: hospitalization	R: Probably Not Related A: Acute Liver Failure Y: 1,2
EPOE-10-01 Treatment Epoetin Hospira	11084-0060 54/W/F	Yes	S: Infections and infestations P: Infectious peritonitis V: Acute peritonitis	S: (b) (6) (183) E: (b) (6) (183)	O: Fatal S: Severe A: Not Applicable X: None	R: Not Related A: Diagnostic Colonoscopy Y: 1

Study Period Treatment	Subject ID Age/Race/Sex	Treatment -Emergent (Yes/No)	S: System Organ Class P: Preferred Term V: Verbatim Term	S: Start Date (Rel. Day) E: Stop Date (Rel. Day)	O: Outcome S: Severity A: Action Taken with Study Drug X: Other Action Taken	R: Relationship[a] A: Alternative Etiology Y: Seriousness Criteria[b]
EPOE-10-01 Treatment Epoetin Hospira	11100-0270 74/W/F	Yes	S: Renal and Urinary Disorders P: Azotaemia V: Uremia	S: (b) (6) (171) E: (b) (6) (171)	O: Fatal S: Severe A: Dose not changed X: None	R: Not Related A: Hepatic Failure Y: 1
EPOE-10-01 Treatment Epoetin Hospira	11100-0352 71/W/M	Yes	S: Renal and Urinary Disorders P: Azotaemia V: Uremia	S: (b) (6) (74) E: (b) (6) (74)	O: Fatal S: Severe A: Dose not changed X: None	R: Not Related A: Hepatic Failure Y: 1,2
EPOE-10-01 Treatment Epogen	11026-0097 55/B/M	Yes	S: Cardiac Disorders P: Cardiac Arrest V: Cardiac Arrest	S: (b) (6) (15) E: (b) (6) (15)	O: Fatal S: Severe A: Drug withdrawn X: Treatment required	R: Not Related A: History Of Myocardial Infarction Y: 1
EPOE-10-01 Treatment Epogen	11102-0290 53/B/M	Yes	S: Cardiac Disorders P: Cardio-Respiratory Arrest V: Death Due To Acute Cardiorespiratory Arrest	S: (b) (6) (26) E: (b) (6) (26)	O: Fatal S: Severe A: Drug withdrawn X: None	R: Not Related A: Congestive Heart Failure Y: 1
EPOE-10-01 Treatment Epogen	11123-0371 63/W/F	Yes	S: Cardiac Disorders P: Cardiac Arrest V: Cardiac Arrest	S: (b) (6) (82) E: (b) (6) (83)	O: Fatal S: Severe A: Not applicable X: Treatment required	R: Not Related A: Post Surgical Anesthesia Complication Y: 1

Study Period Treatment	Subject ID Age/Race/Sex	Treatment -Emergent (Yes/No)	S: System Organ Class P: Preferred Term V: Verbatim Term	S: Start Date (Rel. Day) E: Stop Date (Rel. Day)	O: Outcome S: Severity A: Action Taken with Study Drug X: Other Action Taken	R: Relationship[a] A: Alternative Etiology Y: Seriousness Criteria[b]
EPOE-10-01 Treatment Epogen	13050-0138 49/W/M	Yes	S: Cardiac Disorders P: Cardio-Respiratory Arrest V: Death - Cardiopulmonary Arrest	S: (b) (6) (155) E: (b) (6) (155)	O: Fatal S: Severe A: Not applicable X: None	R: Not Related A: Cardiac Arrest Of Unknown Etiology Y: 1,3
EPOE-10-01 Treatment Epogen	14011-0445 64/W/F	Yes	S: Cardiac Disorders P: Cardiac Arrest V: Cardiac Arrest	S: (b) (6) (72) E: (b) (6) (72)	O: Fatal S: Severe A: Not applicable X: None	R: Not Related A: Hx Of Diabetes Type 2, Coronary Artery Disease, Hypertension Y: 1
EPOE-10-01 Treatment Epogen	14014-0067 53/W/M	Yes	S: Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps) P: Lung Cancer Metastatic V: Deterioration Of Lung Cancer With Metastatic Disease	S: (b) (6) (92) E: (b) (6) (97)	O: Fatal S: Severe A: Not applicable X: Other: Hospitalized	R: Not Related A: Smoking Y: 1,4
EPOE-10-01 Treatment Epogen	15009-0558 70/W/F	Yes	S: Cardiac disorders P: Angina pectoris V: Cardiac pain - right shoulder and scapula	S: (b) (6) (181) E: (b) (6) (187)	O: Fatal S: Severe A: Not applicable X: Treatment required	R: Not related A: Long standing medical history of cardiac disease; SAE onset prior to first dose LTSS Study Drug Y: 1,2,4

During the Titration (Stabilization) Period of Study EPOE-10-13, SAEs resulting in death occurred in 3 subjects in the Epoetin Hospira treatment group and 1 subject in the Epogen treatment group.

Study Period Treatment	**Subject ID Age/Race/Sex**	**Treatment -Emergent (Yes/No)**	**S: System Organ Class P: Preferred Term V: Verbatim Term**	**S: Start Date (Rel. Day) E: Stop Date (Rel. Day)**	**O: Outcome S: Severity A: Action Taken with Study Drug X: Other Action Taken**	**R: Relationshipa A: Alternative Etiology Y: Seriousness Criteriab**
EPOE-10-13 Titration Epoetin Hospira	23004-0166 49/B/F	Yes	S: General Disorders and Administration Site Conditions P: General Physical Health Deterioration V: Multifactorial Functional Decline - No Further Information	S: (b) (6) (81) E: (b) (6) (81)	O: Fatal S: Severe A: Not Applicable X: None	R: Not Related A: Hypertension, Diabetes Mellitus, End Stage Renal Disease, Congestive Heart Failure Y: 1
EPOE-10-13 Titration Epoetin Hospira	23041-0251 68/W/M	Yes	S: Cardiac Disorders P: Cardiac Arrest V: Cardiac Arrest	S: (b) (6) (59) E: (b) (6) (62)	O: Fatal S: Severe A: Drug Withdrawn X: Other: CPR Given In Hospital	R: Not Related A: Complete Heart Block Y: 1,2,4
EPOE-10-13 Titration Epoetin Hospira	24005-0245 55/W/F	Yes	S: Infections and Infestations P: Sepsis V: Septicemia	S: (b) (6) (19) E: (b) (6) (57)	O: Fatal S: Severe A: Not Applicable X: Treatment Required	R: Not Related A: Ischemic Bowel/Necrotizing Fasciitis Y: 1,4
EPOE-10-13 Titration Epogen	23012-0134 61/B/M	Yes	S: Cardiac Disorders P: Acute Myocardial Infarction V: Acute Myocardial Infarction	S: (b) (6) (40) E: (b) (6) (40)	O: Fatal S: Severe A: Drug Withdrawn X: None	R: Not Related A: MI Y: 1

Note: W = White; B = Black or African-American; A = Asian; O = Other; M = Male; F = Female.
Note: Verbatim terms coded using MedDRA version 14.1.
Note: For Start and Ending Dates, Rel. Day = Relative Day. Relative Day = date of assessment – date of first dose of study drug if the assessment date is prior to the date of first dose in the nominal treatment period. If the assessment date is after the date of first dose in the nominal treatment period then Relative Day = date of assessment – date of first dose of study drug + 1.
[a] Relationship to study drug is indicated by the Investigator.
[b] 1=Results in Death; 2=Life threatening; 3=Results in persistent or significant disability/incapacity; 4=Requires or prolongs hospitalization; 5=Congenital abnormality/birth defect; 6=Important medical event requiring medical or surgical intervention to Prevent Serious Outcome.

Table 47. Listing of Subjects with Treatment-Emergent Adverse Events of Special Interest of Thromboemolic Events (Combined Randomized Studies)

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
Study EPOE-10-13 (Enrolled Population) Treatment Epoetin Hospira						
EPOE-10-13/ 23015 0050*	Cerebrovascular Events / Thromboembolic Events	Transient Ischaemic attack	No	Yes	Hypercholesterolemia; previous myocardial infarction, previous transient ischemic attack and peripheral vascular disease	11.3
EPOE-10-13/ 23012-0116	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	History of intermittent arterio-venous clotted graft; deep venous thrombosis	10.5
EPOE-10-13/ 23016 0088	Thromboembolic Events	Vascular graft thrombosis	No	No	BMI >30; age of graft >12 months and stenosis of the access	10.3
	Thromboembolic Events	Vascular graft thrombosis	No	No		10.3
	Thromboembolic Events	Vascular graft thrombosis	No	No		10.3
EPOE-10-13/ 24033-0151	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	BMI >30; history of intermittent arterio-venous fistula stenosis	9.2
EPOE-10-13/ 25002-0280	Thromboembolic Events	Vascular graft thrombosis	No	No	History of deep venous thrombosis; history of intermittent catheter site infection	9.5
	Thromboembolic Events	Vascular graft thrombosis	No	No		8.7
	Thromboembolic Events	Vascular graft thrombosis	No	No		8.0
Study EPOE-10-13 (Enrolled Population) Treatment: Epogen						
EPOE-10-13/ 24026-0261	Myocardial Infarction / Thromboembolic Events	Myocardial infarction	No	Yes	Hyperlipidemia and coronary artery disease with previous myocardial infarct	11.7
	Myocardial Infarction / Thromboembolic Events	Myocardial infarction	No	No		9.5
EPOE-10-13/ 24024-0177	Cerebrovascular Events / Thromboembolic Events	Vertebral artery occlusion	No	No	Concomitant left internal jugular catheter	9.2
EPOE-10-13/ 24009 0200	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	None reported	9.0
EPOE-10-13/ 24011-0231	Thromboembolic Events	Arteriovenous fistula occlusion	No	Yes	None reported	10.0
	Thromboembolic Events	Vascular graft thrombosis	No	Yes		9.1
EPOE-10-13/ 24017-0115	Thromboembolic Events	Vascular graft thrombosis	No	No	None reported	10.0

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
EPOE-10-13/ 24020-0058	Thromboembolic Events	Vena cava thrombosis	No	Yes	Concomitant SAE of non –small cell lung cancer	10.6
EPOE-10-13/ 24024-0177	Thromboembolic Events	Jugular vein thrombosis	No	Yes	Prior non-treatment-emergent AE of jugular vein thrombosis	9.2
	Thromboembolic Events	Superior vena cava syndrome	No	No		8.2
	Thromboembolic Events	Venous occlusion	No	No		10.1
EPOE-10-13/ 24026-0261	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	None reported	10.4
EPOE-10-13/ 25003-0247	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	None reported	10.0
Study EPOE-10-01 (Enrolled Population) Treatment: Epoetin Hospira						
EPOE-10-01/ 11033-0086	Myocardial Infarction / Thromboembolic Events	Acute myocardial infarction	No	No	Previous myocardial infarction	8.6
EPOE-10-01/ 11100-0326	Myocardial Infarction / Thromboembolic Events	Myocardial infarction	No	No	Type 2 diabetes mellitus; hypercholesterolemia; hypertension; congestive heart failure	11.1
EPOE-10-01/ 14028-0013	Myocardial Infarction / Thromboembolic Events	Acute myocardial infarction	No	Yes	Congestive heart failure	9.2
EPOE-10-01/ 14045-0467	Myocardial Infarction / Thromboembolic Events	Acute myocardial infarction	No	Yes	Hypertension; congestive heart failure; coronary artery disease	11.8
EPOE-10-01/ 11033-0086	Cerebrovascular Events / Thromboembolic Events	Embolic stroke	No	No	History of stroke 2006. History of myocardial infarction. Sepsis with development of disseminated intravascular coagulation concomitant with this event	8.6
EPOE-10-01/ 14011-0194	Cerebrovascular Events / Thromboembolic Events	Cerebrovascular accident	No	Yes	Diabetes, hyperlipidemia	9.5
EPOE-10-01/ 11003-0561	Thromboembolic Events	Arteriovenous fistula occlusion	No	No	Aged arteriovenous fistula >3 years	10.0
EPOE-10-01/ 11015-0232	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	History of clotted access 2010; BMI >30 kg/m^2	9.3

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
EPOE-10-01/ 11016-0571	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	None	9.4
EPOE-10-01/ 11033-0086	Thromboembolic Events	Disseminated intravascular coagulation	No	No	Sepsis (previous history of methicillin-resistant Staphylococcus aureus bacteremia)	8.6
EPOE-10-01/ 11083-0148	Thromboembolic Events	Device occlusion	No	No	History of right internal jugular vein dilation	10.0
EPOE-10-01/ 11100-0270	Thromboembolic Events	Vascular graft thrombosis	No	Yes	History of clotted access in 2011; BMI >30 kg/m^2	7.7
EPOE-10-01/ 13003-0386	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	BMI >30 kg/m^2.	10.6
EPOE-10-01/ 13005-0106	Thromboembolic Events	Graft thrombosis	No	No	Pseudoaneurysm May 2012; aged graft >1 year; BMI >30 kg/m^2	12.0
EPOE-10-01/ 13039-0333	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	BMI >30 kg/m^2	10.3
EPOE-10-01/ 13042-0055	Thromboembolic Events	Graft thrombosis	No	No	Recent dialysis access infection; BMI >30 kg/m^2	10.8
EPOE-10-01/ 13042-0073	Thromboembolic Events	Graft thrombosis	No	No	BMI >30 kg/m^2	10.6
EPOE-10-01/ 14007-0041	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	Verbatim adverse event description is “thrombophlebitis left upper arm arteriovenous fistula;” history of concomitant trauma to left forearm; BMI >30 kg/m^2	10.3
EPOE-10-01/ 14011-0422	Thromboembolic Events	Arteriovenous fistula occlusion	No	No	Fistula aneurysm and aged fistula >3 years	8.0
EPOE-10-01/ 14011-0453	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	BMI >30 kg/m^2	9.9
EPOE-10-01/ 14040-0003	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	BMI >30 kg/m^2	10.5
EPOE-10-01/ 14052-0110	Thromboembolic Events	Vascular graft thrombosis	No	No	Aged fistulas >3 years	10.0

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
EPOE-10-01/ 14052-0142	Thromboembolic Events	Graft thrombosis	No	No	None reported	9.9
EPOE-10-01/ 14054-0182	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	BMI >30 kg/m^2	10.9
EPOE-10-01/ 14054-0486	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	Aged fistula >3 years	9.2
EPOE-10-01/ 14065-0383	Thromboembolic Events	Vascular graft thrombosis	No	No	None reported	10.7
EPOE-10-01/ 14065-0385	Thromboembolic Events	Deep vein thrombosis	No	Yes	Recent surgical manipulation for change of pacemaker	11.2
EPOE-10-01/ 14065-0537	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	Pseudoaneurysms	10.4
EPOE-10-01/ 14071-0544	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	History of hypercoagulation in 2012; BMI >30 kg/m^2	11.2
EPOE-10-01/ 15009-0527	Thromboembolic Events	Vascular graft thrombosis	No	No	Multiple previous episodes of thrombosed arteriovenous graft; aged graft >1 year	10.9
Study EPOE-10-01 (Enrolled Population) Treatment: Epogen						
EPOE-10-01/ 14065-0465	Myocardial Infarction / Thromboembolic Events	Acute myocardial infarction	No	Yes	History of hypertension and cardiomegaly	12.8
EPOE-10-01/ 15009-0599	Myocardial Infarction / Thromboembolic Events	Acute myocardial infarction	No	Yes	History of coronary disease and coronary artery bypass graft	9.9
EPOE-10-01/ 11005-0093	Cerebrovascular Events / Thromboembolic Events	Cerebrovascular accident	No	Yes	History of diabetes mellitus, malignant hypertension, hyperlipidemia, and previous vascular disease	10.5
EPOE-10-01/ 13062-0159	Cerebrovascular Events / Thromboembolic Events	Transient ischaemic attack	No	Yes	History of stroke and coronary artery disease; morbid obesity	9.3
EPOE-10-01/ 14065-0465	Cerebrovascular Events / Thromboembolic Events	Cerebral ischaemia	No	No	Associated West Niles encephalitis	12.8
EPOE-10-01/ 11001-0025	Thromboembolic Events	Graft thrombosis	No	Yes	One previous episode of graft thrombosis; graft angioplasty and revision; aged graft >1year; BMI >30 kg/m^2	10.9

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
EPOE-10-01/ 11001-0592	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	One previous episode of arteriovenous fistula thrombosis; aneurysm formation at fistula anastomosis; angioplasty due to stenosis	10.5
EPOE-10-01/ 11003-0235	Thromboembolic Events	Deep vein thrombosis	No	No	History of Factor V Leiden deficiency, pulmonary embolism	9.9
EPOE-10-01/ 11015-0466	Thromboembolic Events	Shunt thrombosis	No	No	None reported	9.9
EPOE-10-01/ 11102-0288	Thromboembolic Events	Deep vein thrombosis	No	No	Concomitant with femur fracture (result of fall)	8.6
EPOE-10-01/ 11116-0147	Thromboembolic Events	Pulmonary embolism	No	Yes	Concomitant pneumonia and declot of the AV fistula	9.8
EPOE-10-01/ 13028-0260	Thromboembolic Events	Deep vein thrombosis	No	No	BMI >30 kg/m^2	9.2

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation	Hb Near Time of Thromboembolic Event (g/dL)[b]
			Results in Death	Other SAE[a]		
EPOE-10-01/ 13046-0335	Thromboembolic Events	Coronary artery occlusion	No	No	History of cardiomyopathy, hyperlipidemia,and transient ischemic attack	10.4
EPOE-10-01/ 13050-0357	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	History of transient ischemic attack 1987	9.5
	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No		9.0
EPOE-10-01/ 13062-0154	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	None reported	8.3
EPOE-10-01/ 14009-0312	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	Previous transient ischemic attack; BMI >30 kg/m^2	9.6
	Thromboembolic Events	Thrombosis in device	No	No		10.3
EPOE-10-01/ 14011-0445	Thromboembolic Events	Arteriovenous fistula thrombosis	No	Yes	Previous episode of clotted arteriovenous graft; BMI >30 kg/m^2	10.3
EPOE-10-01/ 14014-0067	Thromboembolic Events	Arteriovenous fistula thrombosis	No	No	Active non-small cell lung carcinoma with metastases BMI >30 kg/m^2	8.7
EPOE-10-01/ 14052-0273	Thromboembolic Events	Vascular graft thrombosis	No	No	Aged arteriovenous graft >1 year	10.8
EPOE-10-01/ 15009-0599	Thromboembolic Events	Thrombophlebitis superficial	No	No	History of 3x arteriovenous graft thrombosis	8.1

[a] Other SAE did not result in death.
[b] Hb value within 14 days prior to onset of event, or if resulted in death the most recent available Hb value prior to the start date if >14 days.
Note: * indicates subject whose randomized treatment (Enrolled Population) is different than the actual treatment received (Safety Population)

Table 48. Listing of Subjects with Treatment-Emergent Adverse Events of Special Interest of Hypertension (Combined Randomized Studies)

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
Study EPOE-10-13 (Enrolled Population) Treatment: Epoetin Hospira					
EPOE-10-13/ 24028-0061	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-13/ 25003-0308	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-13/ 23015-0050*	Hypertension	Hypertension	No	No	History of hypertension; concurrent intractable vomiting unable to take oral medications
Study EPOE-10-13 (Enrolled Population) Treatment: Epogen					
EPOE-10-13/ 24031-0152*	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-13/ 21012-0042	Hypertension	Hypertension	No	No	History of hypertension
	Hypertension	Hypertension	No	No	
EPOE-10-13/ 24031-0086	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-13/ 25003-0274	Hypertension	Hypertension	No	No	History of hypertension

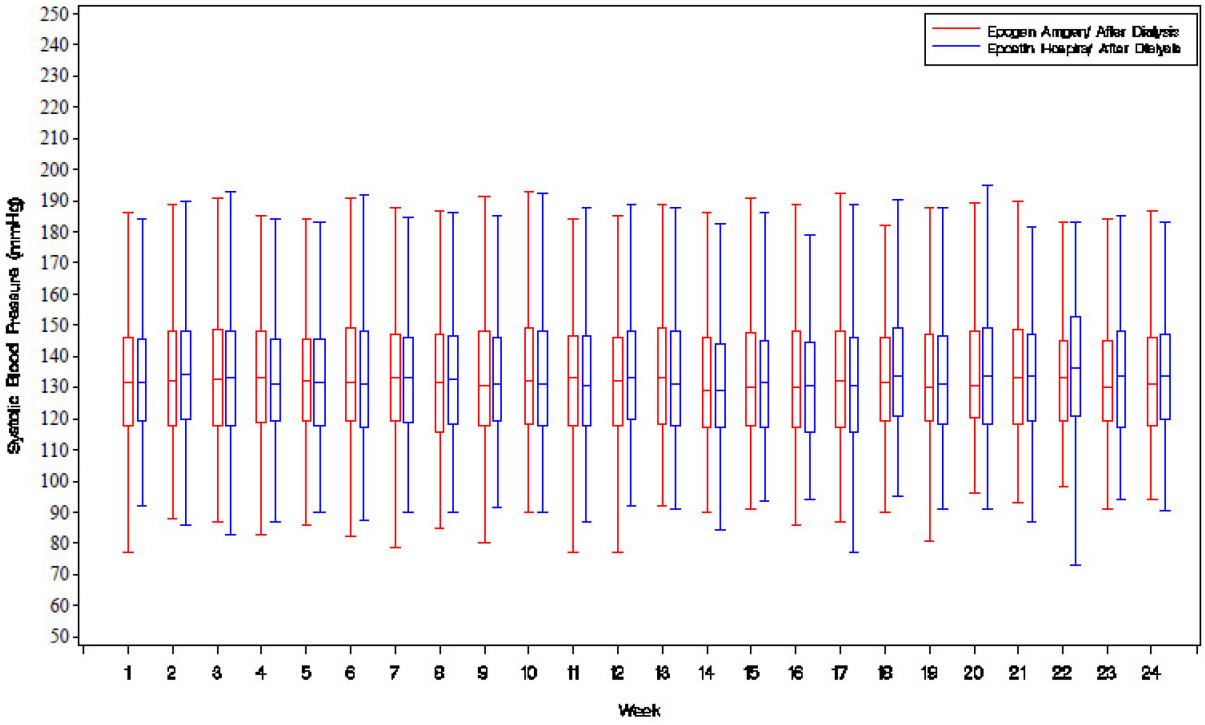
Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
Study EPOE-10-01 (Enrolled Population) Treatment: Epoetin Hospira					
EPOE-10-01/ 11005-0122	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 11014-0071	Hypertension	Blood pressure increased	No	No	History of hypertension
EPOE-10-01/ 11021-0428	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 11084-0061	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 11095-0478	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 13031-0528	Hypertension	Hypertension	No	Yes	History of hypertension
EPOE-10-01/ 13042-0055	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 13042-0144	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 13062-0217	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14011-0422	Hypertension	Procedural hypertension	No	No	History of hypertension
EPOE-10-01/ 14014-0284	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14014-0407	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14014-0408	Hypertension	Hypertension	No	No	History of hypertension
	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14028-0011	Hypertension	Hypertensive crisis	No	Yes	History of hypertension on clonidine since 2010
EPOE-10-01/ 14045-0467	Hypertension	Hypertension	No	No	History of hypertension; congestive heart failure
	Hypertension	Hypertension	No	No	
EPOE-10-01/ 14045-0524	Hypertension	Hypertension	No	No	History of hypertension

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
EPOE-10-01/ 14052-0142	Hypertension	Hypertension	No	No	Hypertension on clonidine
EPOE-10-01/ 14054-0182	Hypertension	Hypertension	No	No	None reported
EPOE-10-01/ 14065-0537	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14071-0413	Hypertension	Hypertension	No	Yes	History of hypertension
EPOE-10-01/ 15005-0482	Hypertension	Blood pressure increased	No	No	History of hypertension
	Hypertension	Blood pressure increased	No	No	
	Hypertension	Hypertension	No	No	
EPOE-10-01/ 15005-0518	Hypertension	Hypertension	No	No	History of hypertension
Study EPOE-10-01 (Enrolled Population) Treatment: Epogen					
EPOE-10-01/ 11005-0095	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 11015-0056	Hypertension	Hypertension	No	Yes	History of hypertension
EPOE-10-01/ 11026-0045	Hypertension	Hypertension	No	Yes	History of hypertension
	Hypertension	Hypertension	No	Yes	
	Hypertension	Hypertension	No	No	
EPOE-10-01/ 11116-0320	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 13060-0303	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 13062-0162	Hypertension	Hypertensive crisis	No	Yes	History of hypertensive urgency
EPOE-10-01/ 14011-0004	Hypertension	Hypertension	No	No	History of hypertension
	Hypertension	Hypertensive crisis	No	No	
	Hypertension	Hypertensive crisis	No	No	

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
EPOE-10-01/ 14011-0216	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14011-0459	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14040-0446	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14045-0195	Hypertension	Hypertension	No	No	History of hypertension
EPOE-10-01/ 14065-0451	Hypertension	Hypertension	No	No	History of hypertension
	Hypertension	Hypertension	No	No	
	Hypertension	Hypertension	No	No	
	Hypertension	Hypertension	No	No	
	Hypertension	Hypertensive crisis	No	Yes	
EPOE-10-01/ 15005-0491	Hypertension	Hypertension	No	No	History of hypertension
	Hypertension	Hypertension	No	No	
	Hypertension	Hypertension	No	No	

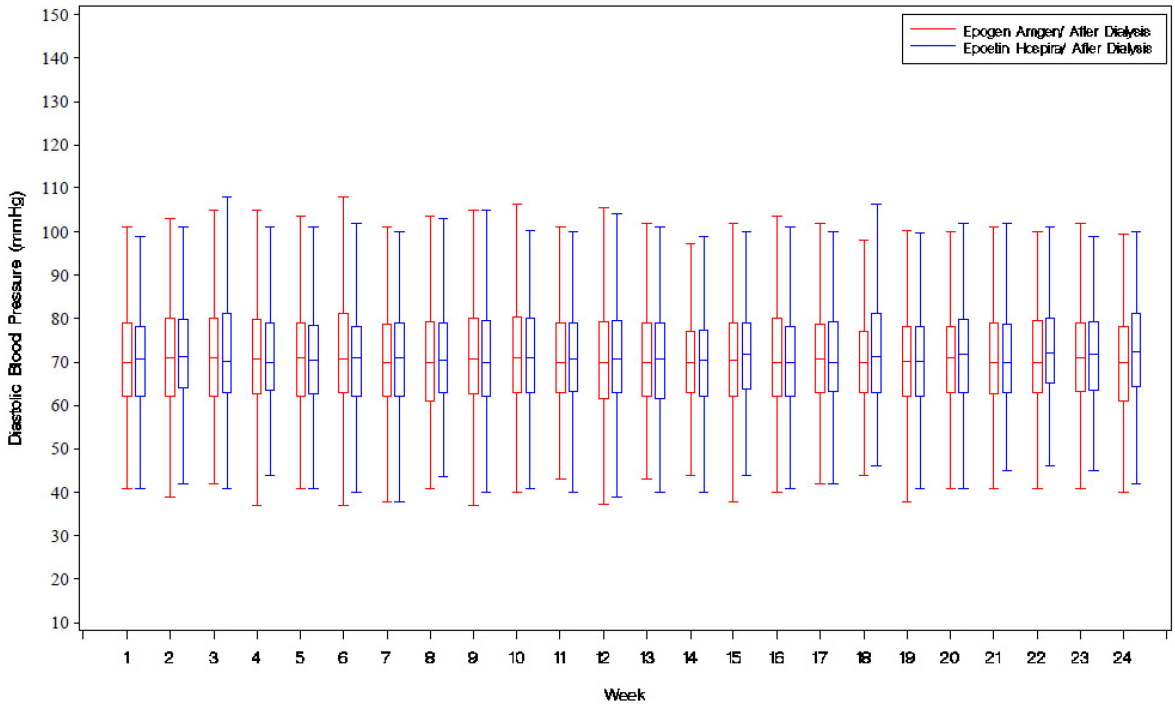
[a] Other SAE did not result in death.
Note: * indicates subject whose randomized treatment (Enrolled Population) is different than the actual treatment received (Safety Population)

Figure 45. Box Plot of Post-Dialysis Systolic Blood Pressure Over Time During the Nominal Treatment Periods for the Combined Randomized Treatment Groups (Safety Population)



Note: The box plot is a box and tails, with box representing the 25th and 75th percentiles, median indicated by the horizontal line, and tail representing the minimum and maximum observed value.

Figure 46. Box Plot of Post-Dialysis Diastolic Blood Pressure Over Time During the Nominal Treatment Periods Combined Randomized Treatment Groups (Safety Population)



Note: The box plot is a box and tails, with box representing the 25th and 75th percentiles, median indicated by the horizontal line, and tail representing the minimum and maximum observed value.

Table 49. Listing of Subjects with Treatment-Emergent Adverse Events of Special Interest of Potential Allergic Reactions (Combined Randomized Studies)

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
Study EPOE-10-13 (Enrolled Population) Treatment: Epoetin Hospira					
EPOE-10-13/ 21012-0109	Potential Allergic Reactions	Lip swelling	No	No	Concurrent trauma secondary to fall
	Potential Allergic Reactions	Swelling face	No	No	
EPOE-10-13/ 24011-0098	Potential Allergic Reactions	Face oedema	No	No	Alternative etiology of fluid overload per Investigator
Study EPOE-10-13 (Enrolled Population) Treatment: Epogen					
EPOE-10-13/ 24031-0152*	Potential Allergic Reactions	Periorbital oedema	No	No	History of edema; alternative etiology reported as fluid retention
EPOE-10-13/ 23015-0223	Potential Allergic Reactions	Face oedema	No	No	History of intermittent facial edema; alternative etiology of concomitant event of brachiocephalic vein stenosis
EPOE-10-13/ 24031-0086	Potential Allergic Reactions	Periorbital oedema	No	No	History of edema; alternative etiology reported as fluid gain
Study EPOE-10-01 (Enrolled Population) Treatment: Epoetin Hospira					
EPOE-10-01/ 11102-0286	Potential Allergic Reactions	Face oedema	No	No	History of edema during dialysis
EPOE-10-01/ 13003-0087	Potential Allergic Reactions	Eye swelling	No	No	History of bilateral ocular implants and as per investigator laser eye surgery
EPOE-10-01/ 13027-0293	Potential Allergic Reactions	Face oedema	No	No	History of intermittent facial edema and lower extremity edema
EPOE-10-01/ 13067-0529	Potential Allergic Reactions	Eye swelling	No	No	History of dialysis induced fluid overload
EPOE-10-01/ 14011-0194	Potential Allergic Reactions	Face oedema	No	No	None reported
EPOE-10-01/ 14052-0234	Potential Allergic Reactions	Face oedema	No	No	None reported
EPOE-10-01/ 14056-0099	Potential Allergic Reactions	Swelling face	No	No	History of fluid overload and generalized edema

Study/ Subject ID	AE of Special Interest Category	Preferred Term	SAE? (Yes)		Pertinent Medical History or Alternative Explanation
			Results in Death	Other SAE[a]	
Study EPOE-10-01 (Enrolled Population) Treatment: Epogen					
EPOE-10-01/ 13005-0392	Potential Allergic Reactions	Face oedema	No	No	None reported
EPOE-10-01/ 13041-0038	Potential Allergic Reactions	Angioedema	No	Yes	Recent start of angiotensin converting enzyme inhibitor
EPOE-10-01/ 13046-0300	Potential Allergic Reactions	Swelling face	No	No	None reported
EPOE-10-01/ 14001-0363	Potential Allergic Reactions	Face oedema	No	No	None reported

a Other SAE did not result in death.
Note: * indicates subject whose randomized treatment (Enrolled Population) is different than the actual treatment received (Safety Population)

Table 50. Listing of Subjects with Positive ADA Result Measured by Updated RIP Assay Using the Supplemental Immunogenicity In-Study Validated Cut Points (Combined Randomized Studies)

Study/ Subject ID	Age/Sex/ Race	Treatment Assignment	Nominal Serial Sample	Final ADA (Anti-rhEPO IgG RIP) Assay Result[a]	ADA (IgG) Titer[b]	Neutralizing Antibody Result[c]	Clinical Comments
EPOE-10-13 21012-0109	46/M/W	Epoetin Hospira	Pre-dose Week 1	positive	1:4	negative	ADA results positive prior to first exposure to Epoetin Hospira. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epoetin Hospira throughout EPOE-10-13.
			Week 16	n/a	NA	NA	
			Follow-up	n/a	NA	NA	
EPOE-10-13 23015-0057	64/F/B	Epoetin Hospira	Pre-dose Week 1	negative	NA	NA	ADA results positive prior to first exposure to Epoetin Hospira. Titers remained stable (between <1:2 to 1:2) throughout clinical course. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epoetin Hospira throughout EPOE-10-13.
			Week 16	positive	<1:2	negative	
			Follow-up	n/a	NA	NA	
EPOE-10-13 21001-0132	65/F/W	Epogen	Week 1 (unscheduled)	positive	<1:2	negative	ADA results negative prior to first exposure to Epogen. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-13.
			Week 16	negative	NA	NA	
			Follow-up	n/a	NA	NA	
EPOE-10-13 24005 0053	26/M/W	Epogen	Pre-dose Week 1	negative	NA	NA	ADA results negative prior to first exposure to Epogen in Maintenance Period. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-13.
			Week 16	positive	<1:2	negative	
			Follow-up	n/a	NA	NA	

Study/ Subject ID	Age/Sex/ Race	Treatment Assignment	Nominal Serial Sample	Final ADA (Anti-rhEPO IgG RIP) Assay Result[a]	ADA (IgG) Titer[b]	Neutralizing Antibody Result[c]	Clinical Comments
EPOE-10-13 24020-0027	52/M/B	Epogen	Pre-dose Week 1	positive	1:2	negative	ADA results negative prior to first exposure to Epogen, then positive prior to first dose in Maintenance Period. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-13.
			Week 16	n/a	NA	NA	
			Follow-up	n/a	NA	NA	
EPOE-10-01 11095-0478	76/M/W	Epoetin Hospira	Pre-dose Week 1	Positive	<1:2	Negative	ADA results positive prior to first exposure to Epoetin Hospira. Titers remained stable throughout clinical course. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epoetin Hospira throughout EPOE-10-01
			Week 12	Positive	1:2	Negative	
			Follow-up or Early Withdrawal	Positive	<1:2	Negative	
			Week 21 (Unsched Visit)	Positive	<1:2	Negative	
EPOE-10-01 14040-0560	72/M/W	Epoetin Hospira	Pre-dose Week 1	Negative	NA	NA	ADA results negative prior to first exposure to Epoetin Hospira. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epoetin Hospira throughout EPOE-10-01.
			Week 12	n/a	NA	NA	
			Week 24	Positive	<1:2	Negative	
			Follow-up	n/a	NA	NA	
EPOE-10-01 14054-0310	71/F/W	Epoetin Hospira	Pre-dose Week 1	Positive	<1:2	Negative	ADA results positive prior to first exposure to Epoetin Hospira. Titers remained stable throughout clinical course. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epoetin Hospira throughout EPOE-10-01
			Week 12	n/a	NA	NA	
			Week 24	Positive	<1:2	Negative	
			Follow-up	n/a	NA	NA	

Study/ Subject ID	Age/Sex/ Race	Treatment Assignment	Nominal Serial Sample	Final ADA (Anti-rhEPO IgG RIP) Assay Result[a]	ADA (IgG) Titer[b]	Neutralizing Antibody Result[c]	Clinical Comments
EPOE-10-01 11045-0276	68/M/W	Epogen	Pre-dose Week 1	Positive	<1:2	Negative	ADA results positive prior to first exposure to Epogen. Titers remained stable throughout clinical course. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-01
			Week 12	n/a	NA	NA	
			Week 24	Positive	<1:2	Negative	
			Follow-up	n/a	NA	NA	
EPOE-10-01 13028-0260	68/F/W	Epogen	Pre-dose Week 1	Positive	<1:2	Negative	ADA results positive prior to first exposure to Epogen. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-01
			Follow-up or Early Withdrawal	Negative	n/a	NA	
EPOE-10-01 14023-0350	49/F/W	Epogen	Pre-dose Week 1	Positive	1:2	Negative	ADA results positive prior to first exposure to Epogen. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-01
			Week 12	n/a	NA	NA	
			Week 24	Negative	NA	NA	
			Follow-up	n/a	NA	NA	
EPOE-10-01 14071-0591	79/F/B	Epogen	Pre-dose Week 1	Negative	NA	NA	ADA results negative prior to first exposure to Epogen. NAb was negative. No evidence of clinical deterioration. No effect on efficacy of Epogen throughout EPOE-10-01.
			Week 12	n/a	NA	NA	
			Week 24	Positive	<1:2	Negative	
			Follow-up	n/a	NA	NA	

a Screening for ADA was conducted using the RIP assay which detects IgG anti-rhEPO binding antibodies. Final ADA result: Negative indicates a negative RIP screening or confirmatory result; Positive indicates a positive screening and confirmatory RIP result. Only subjects with positive confirmatory result are considered positive for Final ADA.

b Final positive ADA samples are titered and the highest titer that remains equal to or above the cut point is reported as the titer.

c Neutralizing antibody assay only performed on samples with a positive Final ADA assay result.

Abbreviations: ADA = anti-drug antibody; B = Black; F = Female; M = Male; n/a = not available; NA = not applicable; RIP = radioimmunoprecipitation; Unsched = unscheduled; W = White

11.3. Definitions of Key Terms

Table 51. Definitions of Key Terms

Term	Definition
Biopotency	The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties. (ICH Q6B)
Cell line	Type of cell population which originates by serial subculture of a primary cell population, which can be banked. (ICH Q5D)
Chromatogram	A graphical experimental result in which the trace of material elution from an analytical column is plotted over time. A chromatogram will typically consist of a number of different peaks; each peak represents a different separated material from the original mixed substance
Comparable	A conclusion that products have highly similar quality attributes before and after manufacturing process changes and that no adverse impact on the safety or efficacy, including immunogenicity, of the drug product occurred. This conclusion can be based on an analysis of product quality attributes. In some cases, nonclinical or clinical data might contribute to the conclusion (ICH Q5E)
Critical quality attribute (CQA)	A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality (ICH Q8[R2])
Deamidation	A chemical reaction in which an amide functional group is removed from an amino acid residue.
Deformulation	Removal of human serum albumin (HSA) from the Epogen/Procrit reference product in order to enable robust comparisons of certain quality attributes.
Deliverable volume	The volume of an injectable drug product as declared on the container closure label that can be accurately extracted and administered to a patient.
Dimer	A species consisting of two protein molecules that are non-covalently self-associated or covalently linked.
Drug Product (DP)	A pharmaceutical product type that contains a drug substance, generally, in association with excipients. (ICH Q6B)
Drug Substance (DS)	The material which is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients including other components such as buffers. (ICH Q6B).
Excipient	An ingredient added intentionally to the drug substance [or drug product] which should not have pharmacological properties in the quantity used. (ICH Q6B)
Fill volume	The amount of drug product that is filled into a single vial, syringe, or other container closure system.
Formulation	The chemical and physical composition of a drug product. (ISPE)
Functional testing	Bioanalytical analyses designed to determine the specific ability or capacity of the product to achieve a defined biological effect.
Glycan	A carbohydrate covalently attached to a protein.
Glycosylation	The covalent addition of carbohydrates to proteins.
Higher order structure	Types of three dimensional structures of a protein, which include secondary, tertiary, and quaternary structures.

Term	Definition
High molecular weight species (HMWS)	Protein species consisting of multiple protein molecules that are non-covalently self-associated or covalently linked.
Host cell protein (HCP)	A process-related impurity consisting of proteins endogenous to the manufacturing cell line
Interchangeability	Designation for a biosimilar that can be expected to produce the same clinical result as the reference product in any given patient; and for a biological product that is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between use of the biological product and the reference product is not greater than the risk of using the reference product without such alternation or switch.
International non-proprietary name (INN)	A unique name, assigned by the World Health Organization, for a pharmaceutical substance or an active pharmaceutical ingredient. INNs are globally recognized are public property.
Limit of quantitation (LOQ)	The lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. (ICH Q2[R1])
Lot	A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. (ICH Q7)
Monomer	A single protein molecule with no covalent or non-covalent, self-associated linkages to another protein molecule.
N-linked glycosylation	The covalent addition of carbohydrates to proteins in which the carbohydrate is attached to the amide group of the side chain of an asparagine residue.
N-Glycan	A glycan covalently attached to a protein at asparagine residues.
nominal treatment period	the 16-week Maintenance Period in Study EPOE-10-13 and/or the 24-week Treatment Period in Study EPOE-10-01
Noncomparative attribute	Protein attributes that are not suitable for comparison in a comparability assessment or a biosimilarity assessment.
O-Glycan	A glycan covalently attached to a protein through serine or threonine residues.
O-linked glycosylation	The covalent addition of carbohydrates to proteins in which the carbohydrate is attached to the hydroxyl group of the side chain of a serine or threonine residue.
Orthogonal (method)	The evaluation of a protein attribute using an additional method that provides different selectivity to the primary method.
Oxidation	The covalent modification of a protein induced either directly by reactive oxygen species or indirectly by reaction with secondary by-products of oxidative stress.
Post-translational modifications	The covalent modification of a protein that occurs after synthesis of the polypeptide is complete. A number of post-translational modifications, such as glycosylation, involve the enzymatic modification of the protein.
Primary structure	The amino acid sequence of a protein.
Product-related impurities	Molecular variants of the desired product (e.g., precursors, certain degradation products arising during manufacture and/or storage) which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety. (ICH Q6B)

Term	Definition
Product-related substances	Molecular variants of the desired product formed during manufacture and/or storage which are active and have no deleterious effect on the safety and efficacy of the drug product. These variants possess properties comparable to the desired product and are not considered impurities. (ICH Q6B)
Reference product	The single biological product licensed under Section 351(a) of the PHS Act against which a biological product is evaluated in a 351(k) application
Residual uncertainty	Term used in stepwise evidence development to describe an observed difference between biosimilar candidate and reference product that may require further exploration
Secondary structure	The regularities in local conformations within a protein molecule, maintained by hydrogen bonds. The most common secondary structures are alpha helices, beta sheets, and random coils.
Sialic acid	A generic term for an N- or O-substituted derivative of neuraminic acid. The most relevant sialic acids are the human form (N-acetylneuraminic acid, or NeuAc), the murine form (N-glycolylneuraminic acid, and NeuGc), and the O-acetylated form of NeuAc ($Neu5,9Ac_2$).
Sialylation	The addition of a sialic acid to an O-linked or N-linked glycan.
Specific Activity (*in vitro* or *in vivo*)	A measure of the biological effect of epoetin used in the biosimilarity assessment, Specific Activity was calculated by dividing the Biopotency (in U/mL) by the Epoetin Content (in µg/mL). Analyses using both *in vitro* Specific Activity and *in vivo* Specific Activity are presented.
Specification	A list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. "Conformance to specification" means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval (ICH Q6B)
Statistical equivalence testing	The most rigorous of the statistical analyses applied during the evaluation of biosimilarity that is applied to Tier 1 attributes. In this analysis, analytical equivalence is concluded if the null hypothesis of *in*equivalence is rejected.
Statistical tier	A construct for evaluating biosimilarity, in which attributes are classified according to their criticality relevant to clinical outcomes. The rigor of the statistical approach for each tier differs as follows: equivalence testing is required for Tier 1 attributes, a quality range approach is used for evaluating Tier 2 attributes, and graphical comparisons are used for Tier 3 attributes.
Tertiary structure	The organization of one or more protein secondary structures into protein domains, which are stabilized by hydrophobic interactions between amino acid side chains.
Totality of evidence	The sum of all of the data and information submitted in a 351(k) application to support a demonstration of biosimilarity. This includes structural and functional characterization, nonclinical evaluation, human PK and PD data, clinical immunogenicity data, and comparative clinical study data.

Term	Definition
Validation	A documented program that provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting pre-determined acceptance criteria (ICH Q7)