



FDA Briefing Document

Oncologic Drugs Advisory Committee Meeting

May 25, 2017

BLA 125545

**“Epoetin Hospira”, a proposed biosimilar to
Epogen/Procrit (epoetin alfa)**

Applicant: Hospira Inc., a Pfizer Company

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We bring the 351(k) BLA for “Epoetin Hospira” with the Applicant’s proposed indications to this Advisory Committee to gain the Committee’s insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.



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Table of Abbreviations

| | |
|---------------------|--|
| ADA | anti-drug antibodies |
| ANCOVA | analysis of covariance |
| ASCO | American Society of Clinical Oncology |
| ASH | American Society of Hematology |
| AUC _{0-∞} | area under curve from time 0 to infinity |
| AUC _{0-t} | area under the concentration-time curve from time zero to time of last nonzero concentration |
| AUC _{0-T} | area under curve from time 0 to last sampling time |
| AUEC _{0-T} | area under effect curve from time 0 to last sampling time |
| BLA | Biologics License Application |
| CHO | Chinese Hamster Ovary |
| CI | confidence interval |
| CKD | chronic kidney disease |
| C _{max} | maximum observed concentration |
| CMS | Centers for Medicare & Medicaid Services |
| dL | deciliter |
| DP | drug product |
| DRISK | Division of Risk Management |
| DS | drug substance |
| EH | “Epoetin Hospira” |
| ELISA | enzyme-linked immunosorbent assay |
| E _{MAX} | maximum effect |
| EPO | erythropoietin |
| ESAs | Erythropoietin Stimulating Agents |
| FDA | Food and Drug Administration |
| FDAAA | Food and Drug Administration Amendments Act |
| FTIR | Fourier Transform infrared spectroscopy |
| g | gram |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practice |
| Hb | hemoglobin |
| HCDNA | host cell DNA |
| HCP | host cell proteins |
| HILIC | hydrophilic interaction liquid chromatography |
| HOS | high order structure |
| HPAEC-PAD | high performance anion exchange chromatography coupled with pulse electrochemical detection |
| HSA | human serum albumin |
| ITT | intent-to-treat |
| IU | International Units |
| IV | intravenous |
| Kg | kilogram |
| L | liter |



| | |
|---------|--|
| LS | least Square |
| m | milli |
| mg | milligrams |
| MS | mass spectrometry |
| MOA | mechanism of action |
| NABs | neutralizing antibodies |
| NCD | National Coverage Determination |
| NeuAc | N-acetylneuraminic acid |
| NeuGc | N-glycolylneuraminic acid |
| OECD | Organization for Economic Co-operation and Development |
| PD | pharmacodynamics |
| pg | picograms |
| PHS | Public Health Service |
| PK | pharmacokinetics |
| PP | per protocol |
| ppm | parts per million |
| PRCA | pure red cell aplasia |
| REMS | Risk Evaluation and Mitigation Strategy |
| RP | reference product; US-licensed Epogen/Procrit |
| RP-UPLC | reverse phase ultra performance liquid chromatography |
| SC | subcutaneous |
| SD | standard deviation |
| SPR | surface plasmon resonance |
| SV-AUC | sedimentation velocity analytical ultracentrifugation |
| SVP | sub-visible particles |
| TEAE | treatment-emergent adverse event |
| TIW | three times a week |
| TK | toxicokinetics |
| µg | micrograms |
| U/mL | units per milliliter |
| US | United States |
| UV CD | ultraviolet circular dichroism |



1 Introduction

Hospira, Inc. (Applicant) has submitted a biologics license application (BLA) under section 351(k) of the Public Health Service Act (PHS Act) for "Epoetin Hospira", a proposed biosimilar to US-licensed Epogen/Procrit¹ (epoetin alfa) (BLA #103234).

Amgen’s BLA #103234 for Epogen/Procrit was initially licensed by FDA on June 1, 1989.

The Applicant is seeking licensure of "Epoetin Hospira" for the same indications as US-licensed Epogen/Procrit:

- 1) 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
- 2) 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/mL
- 3) 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 two additional months of planned chemotherapy
- 4) to reduce the need for allogeneic RBC transfusions among patients with perioperative hemoglobin > 10 to ≤ 13 g/dL who are at high risk for perioperative blood loss from elective, noncardiac, nonvascular surgery.

The purpose of the Oncologic Drugs Advisory Committee meeting is to discuss whether the totality of evidence presented support licensure of “Epoetin Hospira” as a biosimilar to US-licensed Epogen/Procrit. This determination requires the following criteria to be met:

- “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit, notwithstanding minor differences in clinically inactive components, and
- There are no clinically meaningful differences between “Epoetin Hospira” and US-licensed Epogen/Procrit.

2 Background

The Biologics Price Competition and Innovation Act of 2009 (BPCI Act) was enacted as part the Affordable Care Act on March 23, 2010. The BPCI Act created an abbreviated licensure pathway for biological products shown to be “biosimilar” to or “interchangeable” with an FDA-licensed biological product (the “reference product”). This abbreviated licensure pathway under section 351(k) of the Public Health Service (PHS) Act permits reliance on certain existing scientific knowledge about the safety and effectiveness of the reference product, and enables a biosimilar biological product to be licensed based on less than a full complement of product-specific preclinical and clinical data.

¹ For certain figures and tables in this briefing document, the abbreviation “US-Epogen” or “US-Epogen/Procrit” may be used instead of “US-licensed Epogen/Procrit” due to space limitations.



Section 351(k) of the PHS Act defines the terms “biosimilar” or “biosimilarity” to mean that “the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components” and that “there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product.” A 351(k) application must contain, among other things, information demonstrating that the proposed product is biosimilar to a reference product based upon data derived from analytical studies, animal studies, and a clinical study or studies, unless FDA determines, in its discretion, that certain studies are unnecessary in a 351(k) application (see section 351(k)(2) of the PHS Act).

Development of a biosimilar product differs from development of a biological product intended for submission under section 351(a) of the PHS Act (i.e., a “stand-alone” marketing application). The goal of a “stand-alone” development program is to demonstrate the safety, purity and potency of the proposed product based on data derived from a full complement of clinical and nonclinical studies. The goal of a biosimilar development program is to demonstrate that the proposed product is biosimilar to the reference product. While both stand-alone and biosimilar product development programs generate analytical, nonclinical, and clinical data, the number and types of studies conducted will differ based on differing goals and the different statutory standards for licensure.

To support a demonstration of biosimilarity, FDA recommends that applicants use a stepwise approach to developing the data and information needed. At each step, the applicant should evaluate the extent to which there is residual uncertainty about the biosimilarity of the proposed product to the reference product and identify next steps to try to address that uncertainty. The underlying presumption of an abbreviated development program is that a molecule that is shown to be analytically and functionally highly similar to a reference product is anticipated to behave like the reference product in the clinical setting(s). The stepwise approach should start with extensive structural and functional characterization of both the proposed biosimilar product and the reference product, as this analytical characterization serves as the foundation of a biosimilar development program. Based on these results, an assessment can be made regarding the analytical similarity of the proposed biosimilar product to the reference product and, once the applicant has established that the proposed biosimilar meets the analytical similarity prong of the biosimilarity standard the amount of residual uncertainty remaining with respect to both the structural/functional evaluation and the potential for clinically meaningful differences. Additional data, such as nonclinical and/or clinical data, can then be tailored to address these residual uncertainty(-ies).

The ‘totality of the evidence’ submitted by the applicant should be considered when evaluating whether an applicant has adequately demonstrated that a proposed product meets the statutory standard for biosimilarity to the reference product. Such evidence generally includes structural and functional characterization, animal study data, human PK and pharmacodynamics (PD) data, clinical immunogenicity data, and other clinical safety and effectiveness data.



3 Chemistry, Manufacturing, and Controls (CMC)

Executive Summary

“Epoetin Hospira”, a proposed biosimilar to US-licensed Epogen/Procrit was evaluated and compared to US-licensed Epogen/Procrit using multiple orthogonal physicochemical and functional methods. The analytical similarity results and publicly available information support the conclusion that the two products are “highly similar” based on the totality of the analytical data. The data indicate that the amino acid sequences of “Epoetin Hospira” and US-licensed Epogen/Procrit are the same. The results from secondary and tertiary structures and the biological activity analyses met the predefined analytical similarity acceptance criteria. In addition, the stability profile of “Epoetin Hospira” was shown to be similar to that of US-licensed Epogen/Procrit with respect to degradation products and degradation pathways.

Evaluation of the glycosylation profile of “Epoetin Hospira” and US-licensed Epogen/Procrit indicated that both products have similar glycosylation species, with differences in the levels of some glycosylation species between the two products. These differences do not preclude a conclusion that the two products are highly similar because these differences did not impact in vivo biological activity measured using a sensitive assay. As noted in subsequent sections of this briefing book, the additional clinical studies confirm this assessment.

The product related substances and impurities in “Epoetin Hospira” were shown to be similar to that of US-licensed Epogen/Procrit, except for the presence of higher levels (4.5%) of a Cys29-Cys33 trisulfide species in “Epoetin Hospira”. This difference is not expected to have clinical impact. In an earlier version of “Epoetin Hospira”, levels of this species greater than 10% did not result in differences in in vitro and in vivo specific activity. Specific activity is a measure of the inherent biological activity of the molecule. In addition, scientific literature suggests that trisulfide species also form in vivo. As noted in subsequent sections of this briefing book, clinical safety data support this conclusion.

In conclusion, the totality of analytical data support a determination that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit notwithstanding minor differences in clinically inactive components.

Background

Erythropoietin mechanism of action. Endogenous erythropoietin (EPO) is produced primarily in the kidney and stimulates erythropoiesis. The EPO mechanism of action begins with the binding of EPO to the EPO receptor on lineage committed erythroid progenitor cells, primarily found in the bone marrow. This binding initiates signal transduction that leads to the survival, proliferation, and differentiation of erythroid progenitor cells into mature erythrocytes (red blood cells). The later stages of erythropoiesis also depend on availability of iron needed to form the heme complex in hemoglobin. The pharmacodynamic markers commonly used to assess



erythropoiesis are reticulocyte count and hemoglobin levels. Both markers are up-regulated by EPO binding to the EPO receptor and subsequent signal transduction.

The EPO in US-licensed Epogen/Procrit is a 165 amino acid recombinant protein, with an identical amino acid sequence to the isolated natural EPO [US Prescribing Information, Epogen/Procrit]. US-licensed Epogen/Procrit stimulates erythropoiesis by the same mechanism of action as endogenous EPO. The approved indications for US-licensed Epogen/Procrit fall under the general categories of reducing anemia resulting from different disease states and reducing allogeneic blood transfusion [US Prescribing Information, Epogen/Procrit].

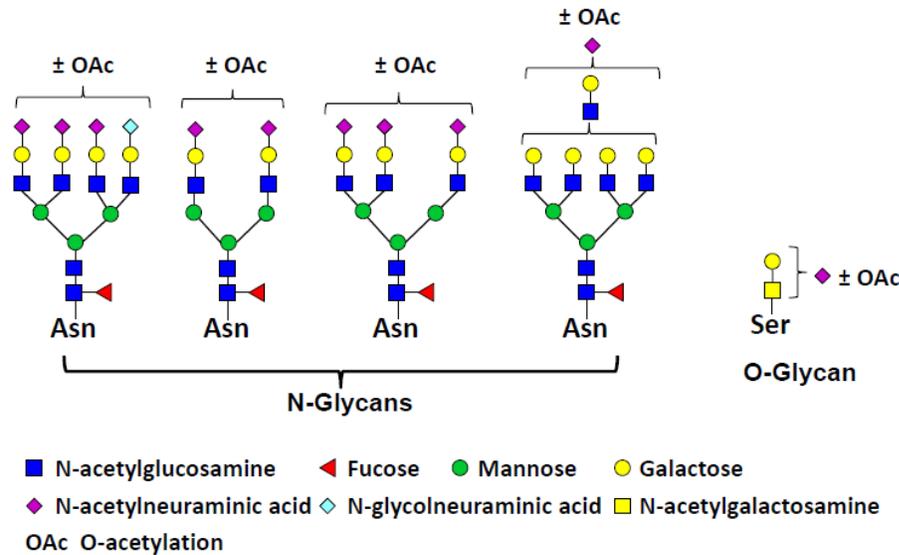
EPO glycosylation and its role in biological activity. Glycosylation is a post-translational modification often necessary for protein function and stability. Isolated human urinary EPO and recombinant EPO produced from mammalian cells are all glycosylated at specific asparagine (N-linked glycosylation) and serine (O-linked glycosylation) residues. The glycosylation sites in endogenous EPO and recombinant EPO are reported to be the same. However, the composition of the glycans, especially the N-linked glycans (also referred to as N-glycans), is complex, heterogeneous, and variable among different EPO products. In recombinant EPO products, these differences are attributed to differences in manufacturing processes [multiple references in Hua et al. 2015].

A schematic representation of some of the possible EPO N- and O-glycan heterogeneity is shown in Figure 1. Typically, each N-glycan contains a fucosylated mannose base to which a variable number of oligosaccharide chains (two to four chains) are attached. These chains are commonly referred to as N-glycan antennarity. The oligosaccharide chains often terminate with a charged sialic acid residue (N-acetylneuraminic acid or NeuAc) attached to a preceding galactose residue. In recombinant EPO, NeuAc is sometimes replaced by other sialic acid variants such as N-glycolylneuraminic acid (NeuGc), the murine counterpart of human NeuAc. Sialic acids may also be acetylated, which adds to the heterogeneity of the glycan. Some oligosaccharide chains are elongated by repeating disaccharide units, known as lactosamine repeats.

The connecting bonds between some monosaccharides may be different and, in cases where incomplete glycan synthesis occurs, high mannose structures can be present. The O-linked glycan is a small and less variable single chain that contains an N-acetylgalactosamine-galactose disaccharide moiety with one or two sialic acid residues that may be acetylated.



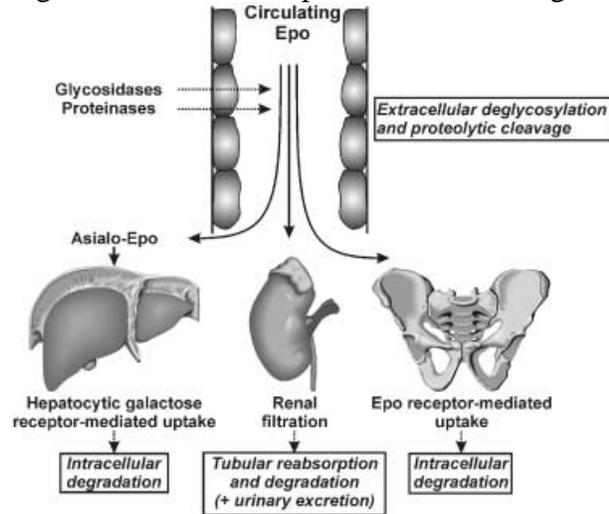
Figure 1. Typical glycan species found in recombinant erythropoietin produced by Chinese hamster ovary cells



Source: Figure drawn by FDA reviewer based on The Consortium for Functional Glycomics glycan nomenclature and literature on EPO glycosylation

The importance of glycosylation in the stability and biological activity of EPO is well documented [Toyoda et al. 2000; Wasley et al. 1991]. N-glycans are proposed to contribute to the pharmacokinetics (PK) of endogenous and recombinant EPO, primarily by increasing the half-life of EPO in circulation [Egrie et al. 2003; Elliot et al. 2004]. The mechanism(s) by which they increase the half-life of circulating EPO remains a subject of discussion; however, several studies suggest that they decrease EPO uptake from circulation and subsequent degradation by the mechanisms shown in Figure 2 [Jelkmann 2002; Misaizu et al. 1995; Chapel et al. 2001]. Notably, several studies suggest that under physiological conditions, EPO receptor mediated-uptake is the major EPO uptake and degradation pathway [Jelkmann 2002; Misaizu et al. 1995; Widness et al. 1996].

Figure 2. Mechanisms postulated in the degradation of circulating erythropoietin



Source: Jelkmann 2002

The role of the individual N-glycan species in EPO biological activity continues to be investigated; however, there is consensus that the terminal sialic acid residues are important for clearance. EPO can contain up to 14 sialic acids per molecule (4 per N-linked and 2 per O-linked glycan) and often result in a mixture of charged isoform species with less than the maximum number of sialic acids.

Studies indicate that in vivo biological activity increases with sialic acid content, often assessed in terms of the quantity of charged isoforms [Egrie et al. 2003; Egrie and Brown, 2001]. One mechanism believed to contribute to clearance of de-sialylated EPO is through uptake by the hepatocytic-galactose receptor (Figure 2), which recognizes non-sialylated galactose residues [Takeuchi et al. 1990]. However, there is a compelling argument for higher sialic acid content potentially increasing the half-life of circulating EPO by decreasing binding to the EPO receptor (EPO receptor-mediated uptake, Figure 2) through electrostatic hindrance [Jelkmann 2002; Darling et al. 2002]. In fact, several studies have shown an inverse relationship between sialic acid content and receptor binding and in vitro biological activity [Jelkmann 2002; Morimoto et al. 1996].

The role of other N-glycan species has also been investigated. Highly branched N-glycans have been postulated to stabilize the EPO conformation [Toyoda et al. 2000] and increase its in vivo biological activity [Takeuchi et al. 1989; Jelkmann 2002; Misaizu et al. 1995] through different mechanisms. However, the role of increased antennarity on in vivo biological activity is sometimes hard to dissociate from the contribution of the subsequent increase in the level of sialylation. With regard to lactosamine repeats, studies have arrived at different conclusions regarding their role of in clearance and biological activity [Cointe et al. 2000; Fukuda et al. 1989].



“Epoetin Hospira” manufacturing. “Epoetin Hospira” is produced by recombinant technology in Chinese Hamster Ovary (CHO) cells transfected with the human EPO gene. The manufacturing process of “Epoetin Hospira” drug substance (DS) consists of various steps intended to isolate and purify EPO. Process-related impurities such as host cell proteins (HCP), host cell DNA (HCDNA), host retrovirus-like particles as well as other process-related impurities specific to the “Epoetin Hospira” manufacturing process are well controlled. Data were provided to demonstrate that the manufacturing process of “Epoetin Hospira” DS is able to reduce the levels of these impurities to very low levels (e.g., ppm for HCP and pg/mg of protein for HCDNA). The ability of “Epoetin Hospira” DS manufacturing process to clear potential viral and other adventitious agent contamination was also demonstrated.

“Epoetin Hospira” drug product (DP) was developed as a liquid, injection, filled in a single use vial in the same strengths (2000 U/mL, 3000 U/mL, 4000 U/mL, 10,000 U/mL and 40,000 U/mL) and for the same indications as those approved for US-licensed Epogen/Procrit. The Applicant is only seeking approval of “Epoetin Hospira” as a biosimilar to the single dose US-licensed Epogen/Procrit presentations and not the multi-dose presentations. The formulation of “Epoetin Hospira” differs from the formulation of US-licensed Epogen/Procrit in inactive ingredients and pH.

The “Epoetin Hospira” DS and DP used in the PK/PD similarity and additional clinical studies used to support a demonstration of biosimilarity were manufactured by the same process as the proposed commercial process. Minor adjustments were made to the proposed DP commercial process after completion of the clinical studies to support similar EPO content between “Epoetin Hospira” and US-licensed Epogen/Procrit. These changes were evaluated and determined to not have an impact on the conclusions from the analytical similarity and clinical studies.

The material used in non-clinical studies was manufactured using different DS and DP manufacturing processes. The Applicant provided data to demonstrate that the “Epoetin Hospira” material used in the non-clinical studies is comparable to that produced by the proposed commercial process, with the exception of EPO content in the DP, which does not impact the safety assessments and conclusions drawn from the non-clinical studies.

The “Epoetin Hospira” DS and DP manufacturing processes are validated and produce product of consistent quality. The controls established for routine manufacture of “Epoetin Hospira” DS and DP meet regulatory expectations.

Analytical Similarity Assessment

Methods. Comparative analytical assessment of “Epoetin Hospira” and US-licensed Epogen/Procrit was used to support a demonstration that “Epoetin Hospira” is “highly similar to US-licensed Epogen/Procrit notwithstanding minor differences in clinically inactive components.” The analytical similarity data discussed in this section for both products were provided by the Applicant. FDA performed an independent analysis of the data including statistical analysis. Prior to analytical similarity assessment, the Applicant assessed and ranked the criticality of EPO quality attributes with respect to biological activity, PK/PD, efficacy, and



safety including immunogenicity. This critical quality attribute (CQA) assessment and ranking informed the analytical similarity exercise, including the statistical analysis used to support comparison of the quantitative attributes. The statistical rigor applied to the analysis of various attributes was based on their criticality and other considerations, in agreement with FDA expectations. Where differences exist between the Applicant’s and FDA conclusions regarding the criticality of an attribute, for example, some glycosylation and charged isoform species, FDA assessed the attribute based on publicly available information, and ensured that the Applicant had an adequate control strategy for the attribute in their product.

Analytical similarity of “Epoetin Hospira” and US-licensed Epogen/Procrit was assessed using the methods listed in Table 1. The methods were validated or qualified at the time of testing and demonstrated to be fit for intended use. The following lots were compared: 35 lots of “Epoetin Hospira” DP, 9 lots of “Epoetin Hospira” DS, all manufactured by the proposed commercial processes, and 54 lots of US-licensed Epogen/Procrit. “Epoetin Hospira” DP and US-licensed Epogen/Procrit lots used in the clinical studies were included in the analytical similarity assessment. The number of lots and strengths analyzed for each product quality attribute was justified by the Applicant based on their assessment of the variability of the analytical method, availability of material, and the impact of the drug product excipients on the analytical method. Where applicable, bracketing across different drug product strengths was used and justified.

Based on the labeled reference product expiration dates and the Applicant’s estimation of their manufacturing date, the US-licensed Epogen/Procrit lots analyzed span approximately 2.6 years and ranged in age from 3 – 12 months when they were analyzed. The “Epoetin Hospira” DP lots analyzed were manufactured between 2011 and 2015 and the DS lots between 2009 and 2013. The “Epoetin Hospira” DP lots included in the analytical similarity exercise were determined by the availability of US-licensed Epogen/Procrit of similar age.

The formulation of US-licensed Epogen/Procrit contains human serum albumin (HSA) [US Prescribing Information, Epogen/Procrit] at concentrations that interfere with the analysis by several analytical methods. The Applicant developed a procedure for removing HSA from the US-licensed Epogen/Procrit to enable comparative analytical testing. The Applicant provided data to demonstrate that the procedure used to remove HSA and the resulting samples (referred to as “deformulated Epogen”) are fit for their intended use. In cases where removal of HSA impacts a product quality attribute, alternative methods were used to compare those quality attributes in samples that contain HSA. Removal of HSA from the US-licensed Epogen/Procrit was not needed for assessment of biological activity and EPO protein content.



Table 1. Quality attributes and methods used to evaluate analytical similarity of “Epoetin Hospira” and US-licensed Epogen/Procrit

| Quality Attribute | Specific Attribute Measured | Method |
|--|--|--|
| Primary Structure | <ul style="list-style-type: none"> • Amino acid sequence • Disulfide mapping • Site of glycosylation and chemical modification | <ul style="list-style-type: none"> • Trypsin peptide map (RP-UPLC with MS, MS/MS) • Glu-C and Lys-C peptide mapping |
| | <ul style="list-style-type: none"> • Free cysteine | <ul style="list-style-type: none"> • RP-HPLC |
| | <ul style="list-style-type: none"> • Molecular weight | <ul style="list-style-type: none"> • De-N-Glycosylated Intact Mass (LC-MS) |
| Post Translational Modification (Glycosylation) | <ul style="list-style-type: none"> • N-glycan site occupancy • N-glycan antennarity • Lactosamine repeats • N- and O-acetylation • N-glycan fucosylation • Total sialic acid content • Sialic acid distribution • Types of sialic acids (NeuAC, NeuGC) • O-site occupancy • O-linked glycan profile • Monosaccharide composition • α-Gal-1,3-Gal • Isoform distribution | <ul style="list-style-type: none"> • HILIC-UPLC-Fluorescence-MS • Anion exchange chromatography • Trypsin peptide map (RP-UPLC-MS) • RP-HPLC • HPAEC-PAD • Capillary Zone Electrophoresis • 2D Chromatography • Enzyme digestion • MS with Isotope labeling |
| Higher Order Structure | <ul style="list-style-type: none"> • Secondary and Tertiary structure | <ul style="list-style-type: none"> • Far- and Near-UV CD • FTIR • Intrinsic Fluorescence • Differential Scanning Calorimetry |
| | <ul style="list-style-type: none"> • Molecular weight of glycosylated protein | <ul style="list-style-type: none"> • SV-AUC |
| Biological activity | <ul style="list-style-type: none"> • In vivo activity, specific activity and total activity per vial | <ul style="list-style-type: none"> • Reticulocyte count in normocythemic mice |
| | <ul style="list-style-type: none"> • In vitro activity, specific activity and total activity per vial | <ul style="list-style-type: none"> • Proliferation of a human leukemic cell line (UT-7 cells) |
| | <ul style="list-style-type: none"> • Receptor binding • Receptor binding kinetics | <ul style="list-style-type: none"> • Competitive receptor binding • SPR |



| Quality Attribute | Specific Attribute Measured | Method |
|--|--|---|
| Drug product attributes | • EPO protein content | • RP-UPLC |
| | • Container fill and deliverable volume | • Volume USP <1> |
| | • Total in vivo and in vitro bioactivity per vial | • See bioassays above |
| | • Particulate matter | • Micro Flow Imaging and Nanoparticle Tracking Analysis |
| Product related Substances and impurities | • Met 54, Trp 64, Trp 88 oxidation | • Lys-C peptide map with UV detection |
| | • Trp 51 oxidation • Asp isomerization (Asp 123, Asp 43) • Deamidation (Asn 147, Asn 47, Gln 86) • Trisulfide at Cys29-Cys33 • Trisulfide at Cys7-Cys161 • Disulfide scrambling | • Trypsin peptide Map (RP-UPLC-MS) |
| | • Dimer and other High Molecular Weight Species | • Quantitative Western Blot • Size Exclusion Chromatography • SV-AUC • Electrophoresis (SDS-PAGE silver stain) |

Source: Table compiled from information provided in Hospira’s 351(k) BLA submission

Analytical Similarity Results

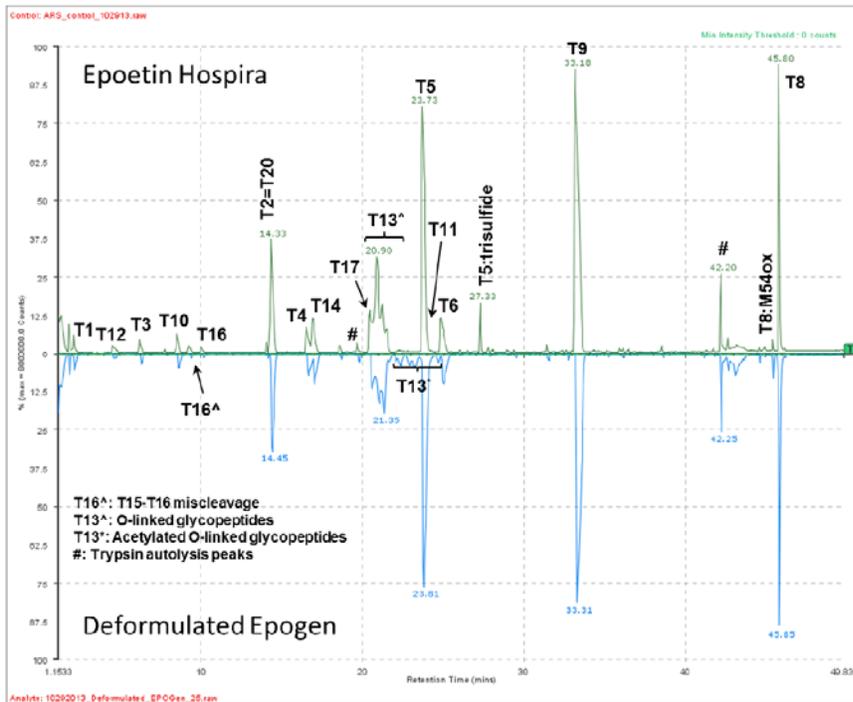
Primary structure. US-licensed Epogen/Procrit EPO is a 165-amino acid protein produced recombinantly from the human EPO gene, and it has an identical amino acid sequence to that of the isolated urinary EPO [US Prescribing Information, Epogen/Procrit]. To support a demonstration that a proposed biosimilar product is highly similar to US-licensed reference product, it is expected that the biosimilar product has the same amino acid sequence (primary structure) as the US-licensed reference product. The Applicant used the analytical methods listed in Table 1 and publicly available information to demonstrate that the amino acid sequences of US-licensed Epogen/Procrit and “Epoetin Hospira” are the same.

Comparative analysis of the amino acid sequence of “Epoetin Hospira” and US-licensed Epogen/Procrit by peptide mapping is shown in the peptide maps in Figure 3. The peptide maps were supported by mass spectrometry data. Both products were analyzed after enzymatic removal of the N-glycans to simplify the chromatographic profile (peptide map). Most peptide peaks are similar between the two products, except peaks T13* and T5:trisulfide, which are not indicative of differences in the amino acid sequence of the products. The differences in peak



T13* are primarily due to heterogeneity in the Ser 126 O-linked glycan. Peak T5:trisulfide corresponds to a trisulfide species that contains an additional sulfur inserted in one of the disulfide bonds of EPO. These trisulfide species are discussed in the “Product Related Substances and Impurities” section of this document.

Figure 3. Representative trypsin peptide maps for “Epoetin Hospira” and US-licensed Epogen/Procrit



Source: Hospira 351(k) BLA submission. The disulfide bonds are located in peptides T5 (Cys29-Cys33 disulfide) and T2=T20 (Cys7-Cys161 disulfide). The glycosylation sites are located in peptides T5 (Asn 24 and Asn 38), T9 (Asn 83), and T13 (Ser 126). “Deformulated Epogen” refers to US-licensed Epogen/Procrit from which human serum albumin was removed from the formulation to enable analytical testing.

Analysis by trypsin peptide mapping showed that 95.2% of the amino acid sequence of the two products is the same. These results were complemented by analysis of “Epoetin Hospira” using Glu-C and Lys-C peptide mapping. The combined peptide mapping results showed that the amino acid sequence of “Epoetin Hospira” is the same as the published amino acid sequence of EPO expressed from the human EPO gene [Lin et al. 1985], as well the sequence of isolated human urinary EPO [Lai et al. 1986].

Human EPO contains two intramolecular disulfide bonds between Cys7-Cys161 and Cys29-Cys33, three N-linked glycosylation sites at Asn 24, Asn 38 and Asn 83, and one O-linked glycosylation site at Ser 126. To confirm the presence of the two intramolecular disulfide bonds and the sites of glycosylation in both products, the Applicant analyzed N-deglycosylated EPO (EPO without the N-linked glycans) from both products using trypsin peptide mapping under non-reducing conditions and mass spectrometry. The presence of disulfide bonds in “Epoetin Hospira” was further confirmed by the absence of free cysteine residues using RP-UPLC. The



results from these studies confirmed the presence of the two disulfide bonds and the expected N-glycosylation sites.

The conclusion that the primary structure for the two products is the same is further supported by the same protein molecular mass obtained by mass spectrometry analysis of N-glycosylated samples from the two products. In addition, the DNA sequence of the “Epoetin Hospira” expression construct indicates that the plasmid encodes the human EPO gene. Restriction analysis of the expression plasmid extracted from the Master Cell Bank and cell cultures maintained beyond the regular production period showed the expected fragments for the EPO expression cassette.

Together, these data provide strong evidence that the primary structures of “Epoetin Hospira” and US-licensed Epogen/Procrit are the same.

Higher order structure (HOS). The secondary and tertiary structures of “Epoetin Hospira” and US-licensed Epogen/Procrit were evaluated by the orthogonal methods shown in Table 1. Secondary structure was compared by Far UV CD spectroscopy and FTIR, two orthogonal assays which provide information about secondary structural elements such as α -helix, β -sheet and random coil structures. The results from Far UV CD and FTIR suggest that the two products have similar secondary structural elements. Comparison of the tertiary structure by Near UV CD spectroscopy suggests that the aromatic residues in “Epoetin Hospira” and US-licensed Epogen/Procrit are in similar structural environments. Similarity of the tertiary structure is supported by overlapping intrinsic fluorescence emission maxima for “Epoetin Hospira” with US-licensed Epogen/Procrit, and similar melting temperatures for the two products measured by Differential Scanning Calorimetry.

Additional structural comparison of the two products was conducted by SV-AUC analysis, which evaluates the rate at which protein molecules move freely in solution in response to a centrifugal force. The sedimentation rate provides information about mass and the shape of a protein. The calculated sedimentation coefficient(s) and molecular weight of the EPO monomer in both products show that the two products are similar with respect to these attributes.

The data derived from the different methods used to assess HOS support a demonstration that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit.

Glycosylation. Comparative analysis of the glycosylation profiles of “Epoetin Hospira” and US-licensed Epogen/Procrit was conducted using the methods listed in Table 1. The N-glycans were analyzed after enzymatic cleavage from the EPO polypeptide chain.

For both products, more than 97% of the EPO protein is glycosylated at positions Asn 24, Asn 38, and Asn 83, and more than 95% is glycosylated at Ser 126. The Applicant’s data showed that the type of glycan species found at each glycosylation site, including the monosaccharides and complex structures such as the number of branched chains and lactosamine repeats is similar between “Epoetin Hospira” and US-licensed Epogen/Procrit. No new glycan species were reported in “Epoetin Hospira”. The glycans found at Asn 38 and Asn 83 mostly contain tetra-

antennary structures, those at Asn 24 are mostly di- and tri-antennary, and the glycan at Ser 126 contains a single glycan chain. Overall, both products contain >50% core fucosylated tetra-antennary tetra sialylated N-glycan structures with zero or one lactosamine repeat.

The amounts of several glycan species are similar between the two products. However, differences in the amounts of some glycan species were observed. For example, although the total content of sialic acids is similar between the two products, differences were observed in the amounts of the acetylated sialic acid species. In addition, distribution of the sialic acids on each glycan and the resulting charged isoforms is slightly different. Some differences were also observed in the relative amounts of each N-glycan antennary structure and the amounts of lactosamine repeats in the two products. Minor structural differences, such as differences in the amounts of glycosylated species do not necessarily preclude a conclusion that the two products are highly similar, provided the Applicant demonstrates that these differences do not result in clinically meaningful differences [FDA Guidance, Scientific Considerations, 2015].

As stated above, differences in EPO glycosylation may affect in vivo biological activity. The Applicant provided data from comparative analysis of in vivo biological activity of “Epoetin Hospira” and the US-licensed Epogen/Procrit. The in vivo biological activity was evaluated using a normocythemc mouse assay, which evaluates reticulocyte production in response to EPO treatment. In vivo biological activity assesses the overall impact of the EPO glycoforms on biological activity. The results show that the two products have similar in vivo activity (see biological activity section). The Applicant provided data to demonstrate that the normocythemc mouse assay used for these studies is sensitive to the glycosylated species under evaluation. Therefore, similar in vivo bioactivity suggests that the differences in glycosylation do not result in an observable net effect on in vivo bioactivity. This conclusion was confirmed by the additional clinical studies.

Erythropoietin Content. “Epoetin Hospira” is a proposed biosimilar to five US-licensed Epogen/Procrit single-use vial strengths, each with a deliverable volume of 1 mL. The manufacturing process of “Epoetin Hospira” DP was designed to target the EPO content in US-licensed Epogen/Procrit. The “Epoetin Hospira” DP control strategy includes criteria to control the EPO content of the DP during routine release and stability testing.

The mean EPO content of the “Epoetin Hospira” DP manufactured by an earlier commercial process, including the lots used in the clinical studies that support the BLA submission, was approximately 3.5% higher than the mean EPO content in US-licensed Epogen/Procrit (Figure 4). This difference, combined with the proposed acceptance criteria to control EPO content in commercial “Epoetin Hospira” DP could potentially allow for larger differences in EPO content between the proposed biosimilar and US-licensed Epogen/Procrit and raised concerns regarding exposure.

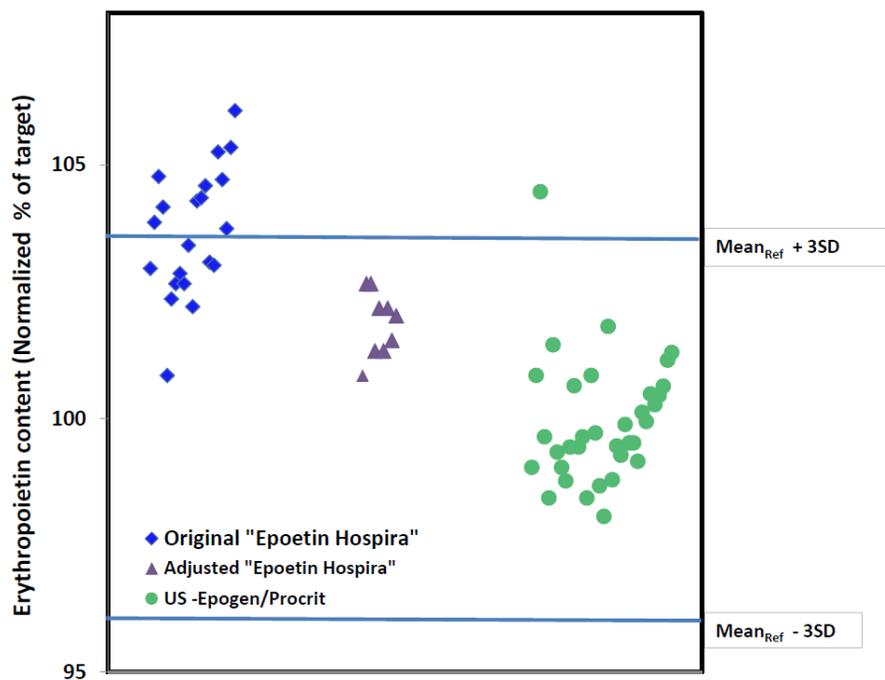
The higher EPO content in “Epoetin Hospira” DP manufactured by this earlier commercial process was determined to be due to a resolvable manufacturing issue. The Applicant agreed to adjust the EPO content in “Epoetin Hospira” DP to more closely match the EPO content of US-licensed Epogen/Procrit DP determined by the Applicant. The revised target EPO content is



based on the mean measured EPO content in US-licensed Epogen/Procrit DP. In addition, the Applicant agreed to tighten the commercial “Epoetin Hospira” DP acceptance criteria for EPO content to a more appropriate range. The Applicant addressed this issue; "Epoetin Hospira" EPO content is within the quality range defined using US-licensed Epogen/Procrit lots, and the data analysis supports a demonstration that the two products are highly similar.

Based on the totality of the evidence, the adjustment in EPO content did not have an impact the conclusions regarding biological activity or the results from the clinical studies.

Figure 4. Erythropoietin content of “Epoetin Hospira” and US-licensed Epogen/Procrit



Source: FDA analysis of data from Hospira 351(k) BLA submission

Biological Activity. The biological activity of the two products was compared using assays that assess various aspects of the EPO mechanism of action, including binding to the EPO receptor, induction of cell proliferation, and production of reticulocytes. Binding to the EPO receptor was assessed by a competitive receptor binding ELISA assay, and receptor binding kinetics were compared by surface plasmon resonance (SPR). The combined ability of EPO to bind to the EPO receptor, elicit signal transduction, and induce cell proliferation was assessed by an in vitro cell-based bioassay using a human leukemic cell line (UT-7), commonly used to evaluate EPO biological activity. The combined effects of receptor binding, signal transduction, and the contribution of glycosylation to the half-life of EPO was evaluated using a compendial-based in vivo normocythemic mouse assay commonly used to assess the in vivo biological activity of EPO. The assay measures reticulocyte production in mice after subcutaneous administration of a given dose of EPO.



The *in vivo* bioassay was the assay primarily used to evaluate the potential impact of the differences in glycosylation between the two products on biological activity. As mentioned earlier, several studies have reported an inverse relationship between receptor binding and *in vitro* cell-based bioactivity with glycosylation, especially sialic acid content. Although, *in vitro* assays are limited by the fact that they cannot measure the contribution of glycosylation on the half-life of the product, these assays provide information about the intrinsic properties of the EPO molecule.

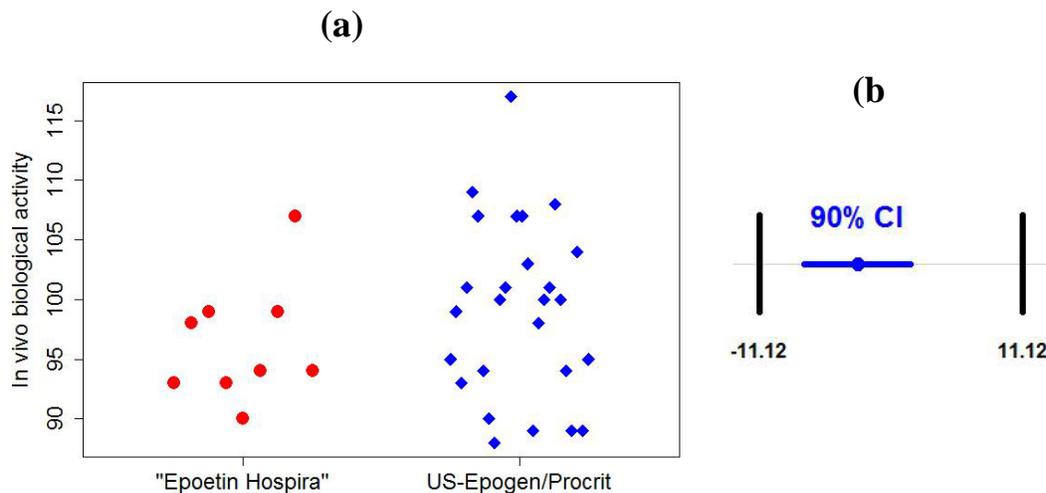
The Applicant’s biological activity data from all assays were expressed as units of activity per volume (e.g., U/ml) or as percentages relative to the Applicant’s in-house reference standard calibrated against an international reference standard for EPO. In addition, the Applicant compared the *in vivo* and *in vitro* specific activity (units of activity per mass of EPO, U/ μ g) of “Epoetin Hospira” and the US-licensed Epogen/Procrit to assess similarity of the intrinsic properties of the EPO molecule.

In vivo biological activity. FDA’s independent analysis of the Applicant’s *in vivo* biological activity data before and after adjustment of EPO content showed that the *in vivo* biological activity of “Epoetin Hospira” is within the biological activity range of the US-licensed Epogen/Procrit lots (Figure 5). Data obtained after adjustment of EPO content is shown in Figure 5 and Table 2 as an example of the analysis. Because *in vivo* biological activity is directly related to the mechanism of action of EPO, these data were subjected to statistical analysis using equivalence testing. *In vivo* biological activity of “Epoetin Hospira” was considered statistically equivalent to that of US-licensed Epogen/Procrit if the 90% confidence interval (CI) of the mean difference between “Epoetin Hospira” and US-licensed Epogen/Procrit is entirely within an equivalence acceptance criterion calculated from the Applicant’s analysis of US-licensed Epogen/Procrit. As per the FDA recommendation provided during product development, the equivalence acceptance criterion was set as $(-1.5 \times \sigma_R, 1.5 \times \sigma_R)$, where σ_R is the variability (standard deviation) calculated by the Applicant from US- Epogen/Procrit data.

The statistical equivalence analysis for *in vivo* biological activity shown in Figure 5 and Table 2 indicates that the 90% CI of the mean difference between the two products is entirely within the equivalence acceptance criteria of $(-11.12, 11.12)\%$. In addition, *in vivo* specific activity (U/ μ g) of “Epoetin Hospira” is within the quality range defined based on the US-licensed Epogen/Procrit. The statistical analyses of the *in vivo* biological activity and *in vivo* specific activity support a conclusion that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit.



Figure 5. (a) Scatter plot of drug product lot values for in vivo biological activity of “Epoetin Hospira” (red solid circles) and US-licensed Epogen/Procrit (blue solid diamonds); (b) Equivalence test plot for in vivo biological activity. The 90% confidence interval for mean difference is represented by a blue segment and equivalence margin by the black vertical lines



Source: FDA analysis of data from Hospira 351(k) BLA submission

Table 2. Statistical analysis of in vivo biological activity

| | # of Lots | Range | Mean (%) | Std. Dev ^a | CV ^b | Mean Diff | 90% CI for Mean Diff. | Equivalence Margin | Statistical Equiv? |
|-------------------|-----------|----------|----------|-----------------------|-----------------|-----------|-----------------------|--------------------|--------------------|
| “Epoetin Hospira” | 9 | 90 - 107 | 96.33 | 5.05 | 0.052 | -2.82 | (-7.29,1.65) | (-11.12,11.12) | Yes |
| US-Epogen/Procrit | 26 | 88 - 117 | 99.15 | 7.41 | 0.075 | | | | |

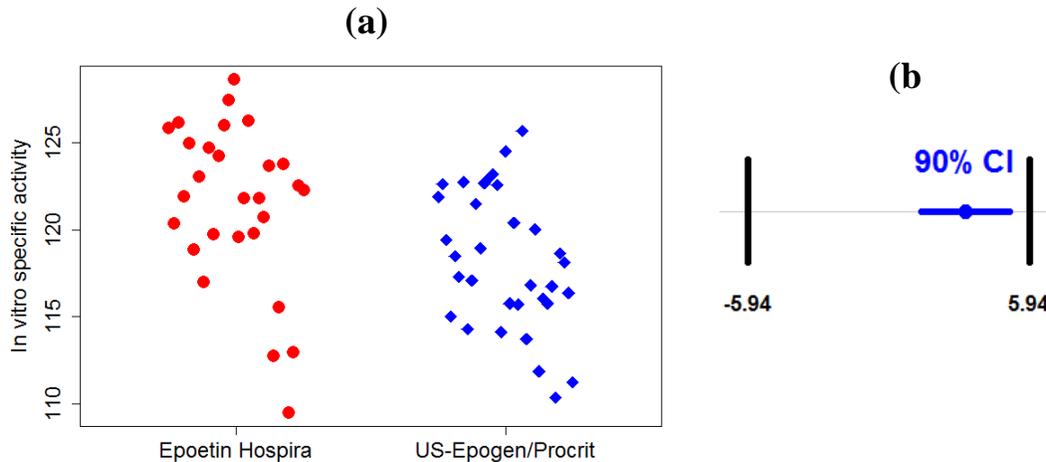
^a Std. Dev is the sample standard deviation for each product;

^b CV: coefficient of variability, computed as the ratio of sample standard deviation to sample mean.

In vitro biological activity. FDA’s independent analysis of the Applicant’s in vitro specific activity data showed that in vitro specific activity of “Epoetin Hospira” before and after EPO content adjustment is within the range of the US-licensed Epogen/Procrit lots (Figure 6 and Table 3). Because in vitro specific activity provides information about an intrinsic property of the EPO protein, these data were subjected to statistical equivalence testing. The in vitro specific activity before and after EPO content adjustment was found to be statistically equivalent (Figure 6 and Table 3). These data support that the intrinsic properties of the molecule that contribute to in vitro activity are similar. In addition, the results of in vitro biological activity (U/mL) after EPO content adjustment are within the $\text{mean}_{\text{ref}} \pm 3\text{SD}$ quality range established based on the Applicant’s analysis of US-licensed Epogen/Procrit. Therefore the statistical analyses of in vitro specific activity and in vitro biological activity support a conclusion that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit.



Figure 6. (a) Scatter plot of drug product lot values for in vitro specific activity of “Epoetin Hospira” (red solid circles) and US-licensed Epogen/Procrit (blue solid diamonds); (b) Equivalence test plot for in vitro specific activity. The 90% confidence interval for mean difference is represented by a blue segment and equivalence margin by the black vertical lines



Source: FDA analysis of data from Hospira 351(k) BLA submission

Table 3. Statistical analysis of in vitro specific activity

| | # of Lots | Range | Mean (%) | Std. Dev ^a | CV ^b | Mean Diff | 90% CI for Mean Diff. | Equivalence Margin | Statistical Equiv? |
|-------------------|-----------|-----------|----------|-----------------------|-----------------|-----------|-----------------------|--------------------|--------------------|
| “Epoetin Hospira” | 28 | 109 - 129 | 121.48 | 4.65 | 0.038 | 3.23 | (1.36, 5.1) | (-5.94, 5.94) | Yes |
| US-Epogen/Procrit | 33 | 110 - 126 | 118.26 | 3.96 | 0.033 | | | | |

^a Std. Dev is the sample standard deviation for each product;

^b CV: coefficient of variability, computed as the ratio of sample standard deviation to sample mean.

Receptor binding and receptor binding kinetics. Comparative assessment of EPO binding to the EPO receptor was conducted using a competitive receptor binding assay and SPR. The activity calculated by comparing the dose response for the products to the Applicant’s internal reference standard and the receptor binding kinetics of the two products including association rate (K_{on}), dissociation rate (k_{off}) and equilibrium dissociation rate (KD) constants support a demonstration that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit

Summary of biological activity. The EPO mechanism of action requires binding of EPO to the EPO receptor and subsequent signaling leading, among other responses, to cell proliferation. The EPO receptor binding and in vitro biological activity results support a conclusion that “Epoetin Hospira” and US-licensed Epogen/Procrit are highly similar. In addition, “Epoetin Hospira” and US-licensed Epogen/Procrit have similar specific biological activity (units of activity per mass of EPO, U/ μ g). This indicates that the intrinsic properties of the two products are similar. The in vivo bioactivity assay measures reticulocyte production and captures the contribution of glycosylation as well as receptor binding and signaling on circulating EPO activity. The results of the in vivo bioactivity assay indicate that the in vivo biological activity is similar between the



two products. These results suggest that the differences in the glycosylation profiles between the two products do not result in observable differences in biological activity in mice.

Based on these results, “Epoetin Hospira” and US-licensed Epogen/Procrit have the same mechanism of action for each of the conditions of use for which licensure is sought. This is further supported by a demonstration that both products have the same amino acid sequence, sites of glycosylation, and similar secondary and tertiary structure.

Product-related substances and impurities. The type and levels of product-related substances and impurities in the two products were assessed quantitatively by the methods listed in Table 1. The results summarized in Table 4 indicate that “Epoetin Hospira” and US-licensed Epogen/Procrit are similar with respect to the type and levels of product related substances and impurities evaluated, except for the presence of higher levels of a trisulfide (Cys29-Cys33) species in “Epoetin Hospira”. Product-related substances and impurities support a demonstration that the two products are highly similar if the same species in similar levels are observed in the two products.

Table 4. Comparison of the type and levels of product-related substances and impurities in “Epoetin Hospira” and US-licensed Epogen/Procrit.

| Species | Supports a Demonstration of Highly Similar? |
|---|---|
| High Molecular Weight Species/Aggregates | Yes |
| Disulfide scrambling species and Dimers | Yes |
| Methionine and Tryptophan Oxidized species (Met 54, Trp 64, Trp 88, Trp 51) | Yes |
| Asparagine isomerization (Asp 123, Asp 43) | Yes |
| Asparagine and Glutamine deamidation (Asn 147, Asn 47, Gln 86) | Yes |
| Trisulfide species (Cys29-Cys33 trisulfide, Cys7-Cys161 trisulfide) | Yes |
| Non-human glycan structure (NeuGc (murine sialic acid), α(1-3) Gal-Gal) | Yes |

Source: FDA analysis of data from Hospira’s 351(k) BLA submission

Cys29-Cys33 trisulfide species. The Cys29-Cys33 trisulfide species are most likely formed during fermentation by insertion of an extra sulfur atom into the Cys29 – Cys33 disulfide bond. Comparative testing of 26 lots of “Epoetin Hospira” DP and 11 lots of US-licensed Epogen/Procrit by trypsin peptide mapping shows that on average, “Epoetin Hospira” contains approximately 4.5% of the Cys29-Cys33 trisulfide species, whereas this species is present at <0.3% in the US-licensed Epogen/Procrit.



Trisulfide species have been reported in some human and recombinant proteins including monoclonal antibodies, human growth hormone (hGH), recombinant truncated interleukin-6 and Cu,Zn superoxide dismutase [Nielsen et al. 2011; Gu et al. 2010; Liu and May. 2012]. The exact chemistry of their formation is not clearly understood. One proposed mechanism in recombinant proteins is through reaction of the disulfide bond with hydrogen disulfide produced during fermentation [Gu et al. 2010; Liu and May. 2012]. A study assessing the impact of these species on the structure, function and stability of monoclonal antibodies suggests that these species are thermally stable under in-vitro conditions, and their presence does not appear to impact monoclonal antigen binding.

In vivo studies in mice show that trisulfide species are converted to disulfide species within the first 24 hours [Gu et al. 2010]. Conclusions that the presence of trisulfide species has no effect on receptor binding were also reached for hGH and vasopressin [Andersson et al. 1996; Moutiez et al. 1997; Thomsen et al. 1994]. However, in the case of interleukin-6, superoxide dismutase, oxytocin and deaminooxytocin, receptor binding and/or biological activity were altered by the presence of trisulfide species [Nielsen et al. 2011 and references therein].

“Epoetin Hospira” and US-licensed Epogen/Procrit have similar in vivo and in vitro specific activities (U/μg), as well as similar receptor binding. In addition, an earlier version of “Epoetin Hospira” containing trisulfide Cys29-Cys33 levels >10% shows similar in vitro and in vivo specific activity as “Epoetin Hospira” with ~4.5% trisulfide Cys29-Cys33. These data suggest that the presence of these species at levels at least twice those found in “Epoetin Hospira” does not appear to have an effect on the intrinsic biological activity of the EPO molecule. Scientific literature suggests that trisulfide species also form in vivo [Gu et al. 2010]. Therefore, this difference is not expected to have clinical impact in this case. This assessment was confirmed by the clinical safety studies.

Other product-related species. In addition to the comparative analysis of the product related substances and impurities listed in Table 4, other product-related species were evaluated in “Epoetin Hospira”, including sequence variants, glycation species, and other glycan variants such as high mannose phosphate structures. None of the product-related species investigated were detected above the quantitation limit of the methods used in “Epoetin Hospira”, except for a C-terminal arginine-166 variant, present at levels of 2% and an N-terminal variant lacking the first two N-terminal amino acids present in levels of ~0.5% in “Epoetin Hospira”.

The C-terminal Arg-166 variant contains an additional Arginine residue at position 166. Based on its DNA sequence, human EPO is reported to contain 166 amino acids, including an Arg in position 166 [Lin et al. 1985; Lai et al. 1986]. The C-terminal Arg-166 variant results from incomplete proteolytic processing of the C-terminal of the mature EPO [Recny et al. 1987]. The second proline from the N-terminal of EPO makes the protein amenable to intramolecular aminolysis to form a diketopiperazine molecule and a protein missing the first two N-terminal amino acids.



The presence of these sequence variants at low levels does not preclude a determination that the two products are highly similar. According to FDA Guidance, the expression construct for a proposed biosimilar product should encode the same primary amino acid sequence as its reference product. However, minor modifications, such as N or C terminal truncations that will not have an effect on safety, purity, or potency, may be justified by the Applicant [FDA Guidance, Quality Considerations, 2015].

As mentioned above, “Epoetin Hospira” has the same amino acid sequence as US-licensed Epogen/Procrit. The C-terminal Arg166 variant, present at a level of 2%, is not expected to impact immunogenicity of the product because this species naturally occurs in humans. In addition, the N-terminal variant present at 0.5% is not expected to impact safety and efficacy, because 1) it is in relatively low quantities, 2) recombinant EPO molecules with N-terminal mutations show full biological activity, and 3) one study suggests that site specific antibodies directed towards the NH₂-terminal region of EPO are not neutralizing [Sue and Sytkowski, 1983].

Sub-visible particles. There is a consensus among immunologists that the immune system may be sensitive to subvisible particles (SVP). Product-related SVP may increase the development of anti-product antibodies. Product-specific immune responses could potentially impact product safety and efficacy [Rosenberg et al. 2012; Carpenter et al. 2008]. SVP and product-specific immune responses were assessed as part of the “Epoetin Hospira” development program. This comparison included clinical studies (discussed in greater detail in the section on Immunogenicity below) and quality attributes, such as sub-visible particles and high molecular weight species. Sub-visible particles are also a concern for capillary occlusion.

The Applicant conducted analytical similarity assessment of particles in the 0.1 – 25 μm range in “Epoetin Hospira” and US-licensed Epogen/Procrit using Microflow imaging and Nanoparticle tracking analysis. The results of the SVP studies indicate that the levels of SVP are similar in the two products.

Comparative stability studies. Comparative stability studies under long term storage conditions, accelerated conditions and stress conditions, including high temperature, forced oxidation, agitation, and photo stress, were conducted on “Epoetin Hospira” and US-licensed Epogen/Procrit. A subset of the methods used in the analytical similarity assessment, including those for EPO content, receptor binding, in vitro biological activity, high order structure, as well as stability indicating methods (e.g., methods to evaluate oxidation, deamidation, Asp isomerization, disulfide related species, and aggregation) were used in the assessment. The results indicate that the two products have similar degradation pathways and degradation products.

CMC Conclusion

“Epoetin Hospira” was evaluated and compared to US-licensed Epogen/Procrit using multiple orthogonal physicochemical and functional methods. The amino acid sequences of “Epoetin Hospira” and US-licensed Epogen/Procrit are the same, which supports the conclusion that the



two products are highly similar. A comparison of the secondary and tertiary structures, as well as the biological activity of “Epoetin Hospira” and US-licensed Epogen/Procrit also supports the conclusion that the two products are highly similar. In addition, the stability profile of “Epoetin Hospira” was shown to be similar to that of US-licensed Epogen/Procrit with respect to degradation products and degradation pathways.

Evaluation of the glycosylation profile of “Epoetin Hospira” and US-licensed Epogen/Procrit indicated that both products have similar glycosylated species, but the amount of some glycosylation species is different between “Epoetin Hospira” and US-licensed Epogen/Procrit. Differences in the amount of glycosylated species could potentially impact clinical efficacy and safety due to differences in exposure. However, the observed differences did not result in observable differences in in vivo biological activity using a sensitive animal assay. As noted in subsequent sections of this briefing book, the observed differences did not have an observable impact on the PK/PD and additional clinical studies.

In “Epoetin Hospira” a Cys29-Cys33 trisulfide species was observed at 4.5% higher levels compared to the US-licensed Epogen/Procrit. This is a product related species that results from insertion of an extra sulfur atom into the Cys29-Cys33 disulfide bond in EPO. Levels of this species greater than 10% did not result in differences in in vitro and in vivo specific activity, which is a measure of the inherent biological activity of the molecule. Scientific literature suggests that trisulfide species also form in vivo. Therefore, this difference is not expected to have clinical impact in this case. As noted in subsequent sections of this briefing book clinical safety data support this conclusion.

In conclusion, the totality of analytical data support the determination that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit notwithstanding minor differences in clinically inactive components.

4 Pharmacology/Toxicology

Executive Summary

“Epoetin Hospira” was compared head-to-head with US-licensed Epogen/Procrit in 13-week animal studies to assess the pharmacodynamics (PD), pharmacokinetics (PK), and toxicity of the products. A meaningful evaluation of the potential differences between “Epoetin Hospira” and US-licensed Epogen/Procrit at three times weekly doses of 150, 450, and 1500/900 IU/kg could only be conducted for the intravenous (IV) route in Beagle dogs. Meaningful comparisons could not be made in the comparative toxicology study in Sprague-Dawley rats evaluating the subcutaneous route (SC) of administration due to reduced exposure and PD activity in US-licensed Epogen-treated rats, which was also associated with a high incidence of neutralizing anti-drug antibody (ADA) development at Week 13.



The nonclinical pharmacology and toxicology data submitted demonstrate similar pharmacodynamic effects in dogs and the same target organs of toxicity in rats and dogs administered “Epoetin Hospira” or US-licensed Epogen/Procrit. Except in instances in rats with lower exposure to US-licensed Epogen/Procrit, exposure to “Epoetin Hospira” was generally lower in animals compared to US-licensed Epogen/Procrit after the dose on Day 1. This may be related to the fact that the “Epoetin Hospira” lot used for the comparative animal toxicology studies had lower protein content (see CMC section for details). Therefore, there are residual uncertainties as to the similarity of the proposed biosimilar to the reference product based on the differences that were observed in the nonclinical data. However, as noted in other sections of this briefing book, these observed differences did not have an observable impact on PK/PD similarity and comparative clinical studies.

Discussion

The animal pharmacology and toxicology studies submitted to the application were assessed with respect to the similarity of toxicities between “Epoetin Hospira” and US-licensed Epogen/Procrit as well as the similarity of other biological responses (e.g., PK/PD) between the two products. The submitted studies were determined to be acceptable for this analysis since recombinant erythropoietin protein is not species-specific, hence making animal toxicology studies relevant to predicting potential effects in humans, and because studies were conducted in compliance with internationally recognized standards (i.e., Organization for Economic Co-operation and Development (OECD)) pertaining to the design and conduct of nonclinical studies according to Good Laboratory Practice (GLP). The nonclinical review found that the particular species, doses, regimens, and duration of exposure selected by the Applicant were based on contemporary methods used to assess the relevant properties of new drugs in addition to our general understanding of erythropoietin pharmacology and toxicology.

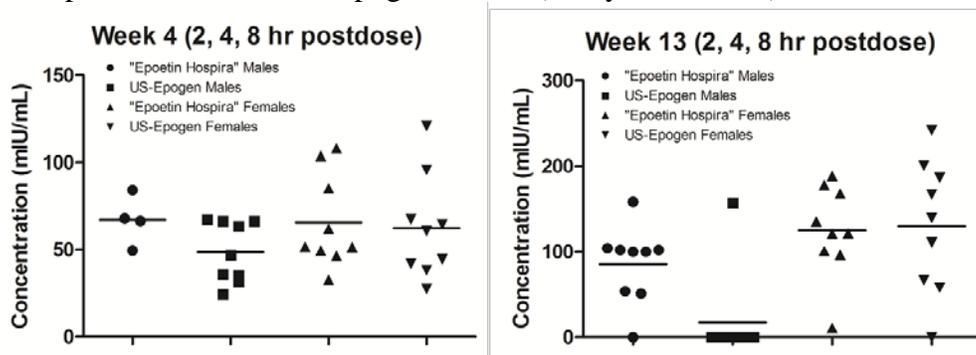
Two GLP-compliant 13-Week comparative toxicology studies were submitted to support the demonstration of biosimilarity of “Epoetin Hospira” to US-licensed Epogen/Procrit. The routes of administration, frequency of dosing, and duration of dosing regimen used in these studies were consistent with the currently approved labeling of US-licensed Epogen/Procrit. Intravenous and SC injection routes of administration with a three times weekly dosing regimen for 13 weeks were assessed in dogs and rats, respectively. The lot of “Epoetin Hospira” drug product used for both 13-Week comparative toxicology studies was formulated based on bioactivity and contained the “Epoetin Hospira” drug substance manufactured at the 400 L “pilot-scale”. This is different from the proposed commercial “Epoetin Hospira” drug substance manufacturing process, which was scaled to 20,000 L and determined the active epoetin component based on a target epoetin protein concentration rather than on the basis of in vivo bioactivity.

The 13-Week SC injection toxicology study with a 4-Week recovery period (ITR-70882) was conducted in Sprague-Dawley rats administered 150, 450 or 1500/900 IU/kg “Epoetin Hospira” or US-licensed Epogen/Procrit three times per week. Mainly at Week 13, very low plasma concentrations were observed in rats treated with US-licensed Epogen/Procrit, except in females treated with 150 IU/kg. Also observed was low PD activity, including no or marginal increases in red blood cell count, hemoglobin, hematocrit, and absolute reticulocytes compared to controls.



Generally, these effects occurred in the presence of neutralizing ADA. Of note, neutralizing ADAs were also present in rats with no correlation with reduced exposure and/or PD activity in either “Epoetin Hospira” or US-licensed Epogen/Procrit treated rats at Week 4. Overall, conclusions regarding the similarity of “Epoetin Hospira” to US-licensed Epogen/Procrit could not be made for SC injection in rats. The individual PD endpoint and exposure data generated with the 150 IU/kg doses of “Epoetin Hospira” or US-licensed Epogen were paid special attention because the 150 IU/kg dose represents approximately the human starting dose, based on the US-licensed Epogen/Procrit package insert.

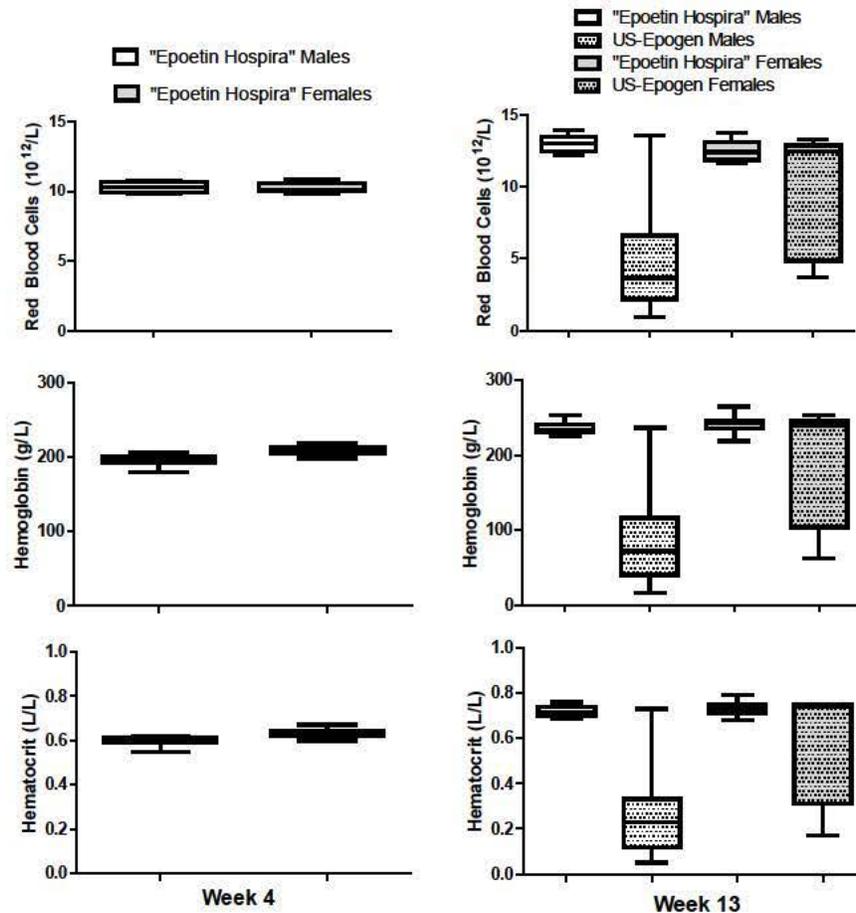
Figure 7. Individual plasma concentrations in rats at sampling time points when maximum plasma concentrations were observed following subcutaneous administration of 150 IU/kg “Epoetin Hospira” or US-licensed Epogen/Procrit (Study ITR-70882)



Source: FDA analysis of data from Hospira 351(k) BLA submission



Figure 8. Pharmacodynamic parameters in rats following subcutaneous administration with 150 IU/kg “Epoetin Hospira” or US-licensed Epogen/Procrit



Source: FDA analysis of data from Hospira 351(k) BLA submission

Table 5. Summary of anti-drug antibody development following subcutaneous administration at 150 IU/kg doses of “Epoetin Hospira” or US-licensed Epogen/Procrit

| Immunogenicity Group Allocated Rats | Males Week 4 | | Females Week 4 | | Males Week 13 | | Females Week 13 | |
|--|-----------------|-----|-------------------|-----|------------------|-----|--------------------|-----|
| | EH | RP | EH | RP | EH | RP | EH | RP |
| Anti-drug Antibody Development (ADA) | | | | | | | | |
| Number of Rats with Binding ADA/Total | 0/6 | 2/6 | 2/6 | 3/6 | 0/6 | 2/5 | 0/3 | 3/4 |
| Number of Rats with Neutralizing ADA/Binding ADA | 0/3 | 2/2 | 3/3 | 3/3 | 0/0 | 2/2 | 0/0 | 3/3 |

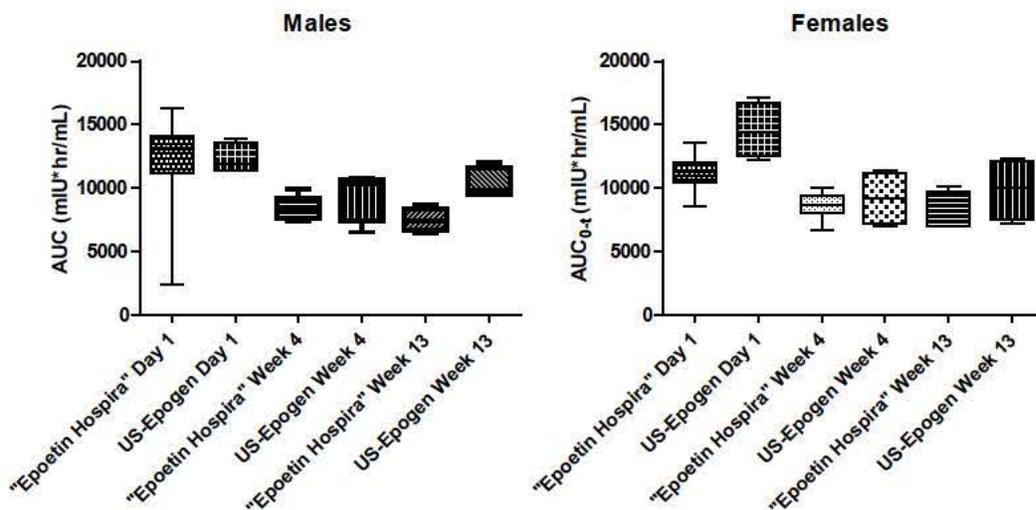
Abbreviations: EH = “Epoetin Hospira”; RP =US-Epogen/Procrit

Source: FDA analysis of data from Hospira 351(k) BLA submission

The 13-Week IV toxicology study with a 4-Week recovery period (ITR-60486) was conducted in Beagle dogs administered 150, 450 or 1500/900 IU/kg “Epoetin Hospira” or US-licensed Epogen/Procrit three times weekly. There was mortality at doses ≥ 450 IU/kg, with more total deaths in “Epoetin Hospira” treated dogs, but more death in US-licensed Epogen/Procrit treated dogs at lower doses. Similar increases in PD endpoints between “Epoetin Hospira” and US-licensed Epogen/Procrit were observed at all doses, with maximum increases nearly achieved at lowest dose of 150 IU/kg for both products. Overall, the organs of toxicity were similar between “Epoetin Hospira” and US-licensed Epogen/Procrit.

Exposures to erythropoietin were measured at several timepoints throughout the 13-Week studies in rats and dogs. Toxicokinetic (TK) parameters (C_{max} and AUC_{0-t}) for “Epoetin Hospira” were slightly lower on all assessment days (1, 26, and 89) and at all doses (up to 40% on Day 89) in dogs and in rats on Day 1. Five dogs developed ADA: one US-licensed Epogen/Procrit treated female at each dose, one US-licensed Epogen/Procrit treated male at 1500/900 IU/kg, and one “Epoetin Hospira” treated female at 1500/900 IU/kg. The majority of these binding ADAs were neutralizing. While the incidence of ADA was lower in the dog study, the onset was similar to the rat study (somewhere between 2 and 4 weeks following the first administered dose).

Figure 9. Exposure (AUC_{0-t}) to erythropoietin in dogs repeat dosed with 150 IU/kg “Epoetin Hospira” or US-licensed Epogen/Procrit (Study ITR-60486)



Source: FDA analysis of data from Hospira 351(k) BLA submission

Pharmacology/Toxicology Conclusion

In summary, the animal studies submitted clearly demonstrated similarity of “Epoetin Hospira” to US-licensed Epogen/Procrit administered IV in terms of pharmacodynamics and toxicity in dogs. There were some differences between “Epoetin Hospira” and US-licensed Epogen/Procrit toxicokinetics in dogs that could be related to the lower protein content in the non-commercial lots of “Epoetin Hospira” used in the toxicology studies; however, the differences were generally



within the range of individual animal variability. The lower PK/PD activity in rats treated SC with US-licensed Epogen/Procrit precluded a determination of similarity for SC administration. The lower PK/PD activity for subcutaneously administered US-licensed Epogen/Procrit groups could be related to ADA development. For example, across the doses tested, less ADA development was observed in “Epoetin Hospira” versus US-licensed Epogen/Procrit treated rats, which had immunogenic human serum albumin in the formulation. Also, more ADA development was observed with the SC versus the IV route of administration and ADA development with long-term repeat SC dosing of recombinant human erythropoietin in rats has been documented previously in published literature [Tillman, HC et al, 2006].

Therefore, from the perspective of pharmacology and toxicology, there are residual uncertainties as to the PK/PD similarity of the proposed biosimilar to the reference product based on the differences that were observed in the animal data (i.e., pharmacodynamics in rats and toxicokinetics in rats and dogs). However, as noted in other sections of this briefing book, these observed differences did not have an observable impact on PK/PD similarity and comparative clinical studies.

5 Immunogenicity

Executive Summary

Immunogenicity for erythropoietin is linked to the development of life-threatening pure red cell aplasia. The incidence of immunogenicity for “Epoetin Hospira” and US-licensed Epogen/Procrit was compared in 3 multiple-dose, parallel-arm studies in 849 patients with chronic kidney disease (EPOE-10-01 and EPOE-10-13) and 129 healthy volunteers (EPOE-14-01). The results indicate similar rates and titers of anti-drug antibodies (ADA) for both products. No neutralizing ADA were detected in any of the clinical studies and no apparent impact of ADA on safety, pharmacokinetic, or pharmacodynamic endpoints were observed. Therefore, the data indicates that there is no increase in immunogenicity risk for “Epoetin Hospira” as compared to reference product US-licensed Epogen/Procrit.

Discussion

Background. Administration of recombinant therapeutic proteins has the potential to induce an unwanted immune response in the patients that can impact the safety, efficacy, and pharmacokinetics of the products. Often, unwanted immune responses to therapeutic biologics are measurable in the form of anti-drug antibodies (ADA) that can be detected in serum following exposure to the drug. Immunogenicity is of particular concern for recombinant EPO products because they are a modified version an endogenous human protein that is present at very low concentrations and has a non-redundant function in erythropoiesis. Subjects receiving recombinant EPO therapy who develop ADA that bind and neutralize endogenous erythropoietin can develop life-threatening Pure Red Cell Aplasia (PRCA).



Applications submitted under section 351(k) of the PHS act contain clinical information aimed at demonstrating the biosimilarity of the proposed biosimilar to the reference product. This information includes “a clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics) that are sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biological product.” To address this, Hospira performed a comparative assessment of ADA development in healthy subjects and patients with chronic kidney disease treated with “Epoetin Hospira” and US-licensed Epogen/Procrit.

Methods. The development of anti-drug antibodies was monitored in each of the “Epoetin Hospira” clinical studies, however three studies were considered to have a design adequate to compare immunogenicity. Studies EPOE-10-01, EPOE-10-13, and EPOE-14-01 have multiple-dose, parallel arm designs that allow for a comparative assessment of immunogenicity between “Epoetin-Hospira” and US-licensed Epogen/Procrit since the ADA are attributable to either the biosimilar or the reference product. The individual study designs, patient populations, treatment, and immunogenicity sampling schedules of these three studies are summarized in Table 6.

Table 6. Immunogenicity sampling in comparative clinical studies for “Epoetin Hospira”

| Study ID | Design | Route | Number | Subjects | Dose | Schedule | Sampling |
|-------------------|----------|---------------|---|----------|----------|--|---|
| EPOE-10-01 | Parallel | Intravenous | "Epoetin Hospira" (N=301) US-Epogen/Procrit (N=304) | CKD | Variable | 1-3 times / week up to 24 weeks | Pre-dose week 1, Week 12, Week 24, Follow up |
| EPOE-10-13 | Parallel | Sub-cutaneous | Titration "Epoetin Hospira"(N=80) US-Epogen/Procrit (N=86) Maintenance "Epoetin Hospira" (N=122) US-Epogen/Procrit (N=122) | CKD | Variable | 1-3 times / week Titration 12-18 weeks Maintenance ce up to 16 weeks | Pre-dose Week 1 titration, End titration, Pre-dose week 1 maintenance, Week 16 maintenance, Follow up |
| EPOE-14-01 | Parallel | Sub-cutaneous | "Epoetin Hospira" (N=66) US-Epogen/Procrit (N=63) | Healthy | 100 U/kg | 3 times / week for 4 weeks | Pre-dose Day 1, Day 12, Day 28 |

Source: Summary based on information from Hospira 351(k) BLA submission

Serum samples were tested for ADA using a tiered strategy as recommended by FDA. A screening assay was used to test all samples. Samples deemed positive in the screening assay were then tested in a confirmatory assay to show that the binding was specific for the product. Samples confirmed positive were titered and assessed for neutralizing activity.



Results. The results of the multiple-dose, parallel-arm clinical studies EPOE-10-01, EPOE-10-13, and EPOE-14-01 are summarized in Table 7.

Table 7. Immunogenicity results for comparative clinical studies for “Epoetin Hospira”

| EPOE-10-01 | | | | |
|---------------------------------|----------|-----------------|--------------------------|-------------|
| | N | Baseline | Treatment-Induced | NAbs |
| “Epoetin Hospira” | 301 | 0.7% | 0.4% | 0.0% |
| US-Epogen/Procrit | 304 | 1.1% | 0.4% | 0.0% |
| EPOE-10-13 | | | | |
| “Epoetin Hospira” (Titration) | 80 | 1.4% | 0.0% | 0.0% |
| US-Epogen/Procrit (Titration) | 80 | 1.3% | 0.0% | 0.0% |
| “Epoetin Hospira” (Maintenance) | 122 | 0.9% | 1.0% | 0.0% |
| US-Epogen/Procrit (Maintenance) | 122 | 1.0% | 0.9% | 0.0% |
| EPOE-14-01 | | | | |
| “Epoetin Hospira” | 66 | 4.5% | 3.0% | 0.0% |
| US-Epogen/Procrit | 63 | 3.2% | 3.2% | 0.0% |

Source: FDA analysis of data from Hospira 351(k) BLA submission

EPOE-10-01. Patients with CKD enrolled in EPOE-10-01 were treated with “Epoetin Hospira” or US-licensed Epogen/Procrit 1-3 times per week for up to 24 weeks. Patients were tested for ADA at baseline, week 12, week 24, and the 28-day follow-up. Prior to treatment (baseline), 0.7% of patients (2 of 269) in the “Epoetin-Hospira” treatment arm were confirmed as positive for ADA versus 1.1% (3 of 265) in the US-licensed Epogen/Procrit treatment arm. The rates of treatment-induced ADA (i.e. patients who were negative at baseline and became positive during the study) were 0.4% for both treatment arms. The ADA titers of the positive samples were 1:40 or less. No samples tested positive for neutralizing antibodies. There was no apparent impact of ADA status on reported adverse events in EPOE-10-01 patients.

EPOE-10-13. EPOE-10-13 consisted of a titration phase and a maintenance phase. Samples for ADA testing were collected at day 0 of the titration period, the end of the titration period, day 0 of the maintenance period, week 16 of the maintenance period, and the 28-day follow-up after the end of treatment. At baseline 1.4% of patients (1 of 71) in the “Epoetin Hospira” arm and 1.3% of patients (1 of 78) in the US-licensed Epogen/Procrit arm were positive for ADA. During the titration period, no patients who were negative at baseline developed ADA. During the maintenance period of the study 0.9% of patients (1 of 109) in the “Epoetin Hospira” arm were positive at baseline and 1.0% of patients (1 of 104) developed treatment emergent ADA after



exposure to “Epoetin Hospira”. In the US-licensed Epogen/Procrit arm of the study, 1.0% of patients (1 of 105) were positive for ADA at baseline and 0.9% (1 of 108) developed treatment-emergent ADA. No neutralizing antibodies were detected in either arm of the study. There was no apparent impact of ADA status on reported adverse events in EPOE-10-13 patients.

EPOE-14-01. Study EPOE-14-01 was a multiple dose, parallel arm study conducted in healthy male volunteers. The study drug was administered as 3 subcutaneous injections (100 U/kg) per week for 4 weeks. Serum samples were collected at baseline, day 12, and day 28 for ADA analysis. In the “Epoetin Hospira” arm 4.5% of subjects (3 of 66) were positive at baseline and 3.0% of subjects (2 of 66) developed treatment emergent ADA. In the US-licensed Epogen/Procrit arm 3.2% of subjects (2 of 63) were positive at baseline and 3.2% (2 of 63) developed treatment-emergent ADA. The titers of the ADA in the positive samples ranged from 1:40 to 1:5120. The highest titers were observed in a subject that received US-licensed Epogen/Procrit. No neutralizing ADA were detected in the “Epoetin Hospira” or US-licensed Epogen/Procrit arms of the study. There was no impact of ADA on PK and PD (hemoglobin level) parameters from Study EPOE-14-01 (data not shown).

Immunogenicity Conclusion

The data support a determination of no clinically meaningful differences in immunogenicity risk between “Epoetin Hospira” and US-licensed Epogen/Procrit.

6 Clinical Pharmacology

Executive Summary

The objectives of the clinical pharmacology program are to evaluate the pharmacokinetic and pharmacodynamic (reticulocyte count and hemoglobin level) similarity between “Epoetin Hospira” and US-licensed Epogen/Procrit.

The Applicant submitted studies EPOE-12-02 and EPOE-14-01 which evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of “Epoetin Hospira” and US-licensed Epogen/Procrit following single and multiple doses, respectively, in healthy subjects. The PD markers were reticulocyte count (Study EPOE-12-02) and hemoglobin level (Study EPOE-14-01).

Study EPOE-12-02 was a randomized, open-label, cross-over study in 81 healthy subjects that evaluated single-dose PK and PD (reticulocyte count) similarity of “Epoetin Hospira” to US-licensed Epogen/Procrit. The study compared the PK, PD (reticulocyte count), safety, and tolerability of single 100 U/kg subcutaneous (SC) dose of “Epoetin Hospira” vs. US-licensed Epogen/Procrit. In this study, the pairwise comparison of “Epoetin Hospira” and US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PK similarity (90% CIs for the ratios of geometric mean of AUC_{0-Inf} , AUC_{0-T} , and C_{MAX} , within 80% to 125% range), thus



establishing the PK similarity. Furthermore, the pairwise comparison of “Epoetin Hospira” or US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PD (reticulocyte count) similarity (90% CIs for the ratios of geometric mean of reticulocyte count $AUEC_{0-T}$ and E_{MAX} within 80% to 125% range), thus establishing the PD similarity.

Study EPOE-14-01 was a randomized, open-label, parallel group study in 129 healthy subjects that evaluated multiple-dose PD (hemoglobin level) similarity of “Epoetin Hospira” to US-licensed Epogen/Procrit. The study compared the PK, PD (hemoglobin level), safety, tolerability, and immunogenicity of 100 U/kg SC three times weekly (TIW) for 4 weeks of either “Epoetin Hospira” or US-licensed Epogen/Procrit. The pairwise comparison of “Epoetin Hospira” or US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PD (hemoglobin level) similarity (90% CIs for the ratios of geometric mean of hemoglobin level $AUEC_{0-T}$ and E_{MAX} within 80% to 125% range), thus establishing the PD similarity.

Overall, the two studies support a demonstration of no clinically meaningful differences in PK and PD between “Epoetin Hospira” and US-licensed Epogen/Procrit.

Discussion

Description of Clinical Pharmacology Studies. The clinical pharmacology studies characterized the PK and PD of “Epoetin Hospira” and US-licensed Epogen/Procrit, following SC administration. A summary of each study, including PK and PD endpoints, is provided below:

- Study EPOE-12-02 was a single-center, randomized, open-label, cross-over study to determine the PK and PD (reticulocyte count) of “Epoetin Hospira” and US-licensed Epogen/Procrit following a single dose of 100 U/kg SC in healthy subjects (N=81). The pre-defined PK endpoints were baseline-adjusted epoetin alfa AUC_{0-T} , AUC_{0-INF} , and C_{MAX} . The pre-defined PD endpoints were reticulocyte count (expressed as a percentage of erythrocytes) $AUEC_{0-T}$ and E_{MAX} . The washout period was 28 days.
- Study EPOE-14-01 was a single-center, randomized, open-label, parallel group study to determine the PK and PD (hemoglobin level) of “Epoetin Hospira” and US-licensed Epogen/Procrit following multiple doses of 100 U/kg SC three times weekly (TIW) for 4 weeks in healthy subjects (N=129). The pre-defined PD endpoint was hemoglobin $AUEC_{0-28d}$ over 28 days. FDA also considers hemoglobin E_{MAX} as a primary PD endpoint. The sponsor also evaluated multiple-dose PK, which FDA considers to be supportive in this application, and the pre-defined PK endpoints were epoetin alfa AUC_{0-48h} and C_{MAX} post-final dose on Day 26.

As described in the FDA Guidance for Industry entitled, “Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product,” a single-dose, randomized study is generally the preferred design for PK similarity assessment. Furthermore, a cross-over study design is recommended for products with a short half-life (e.g., less than 5 days), rapid PD



response, and low incidence of immunogenicity. Conducting the study in healthy subjects is acceptable as it is safe and more sensitive in evaluating product similarity due to lack of potential confounding factors such as underlying disease, concomitant medications, and other factors. For single-dose assessment of PK and PD (reticulocyte count) similarity, a cross-over design is therefore appropriate due to the short half-life, reticulocyte response time and low incidence of immunogenicity for EPO. The 100 U/kg dose tested is relevant as it is one of the approved doses and is in the sensitive portion of the dose-response curve. The single-dose study is considered the pivotal study for evaluating PK similarity by FDA. For multiple-dose assessment of PD (hemoglobin level) similarity, a parallel design is appropriate due to the time it takes to elicit an appreciable hemoglobin response.

Results of Clinical Pharmacology Studies

Study EPOE-12-02

Pharmacokinetic Results

In Study EPOE-12-02, the pairwise comparison of “Epoetin Hospira” and US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PK similarity (90% CIs for the ratios of geometric mean of AUC_{0-120h} , AUC_{0-INF} , and C_{MAX} , within 80% to 125% range) as summarized in Table 8 and depicted in Figure 10.

These data establish the PK similarity between “Epogen Hospira” and US-licensed Epogen/Procrit.

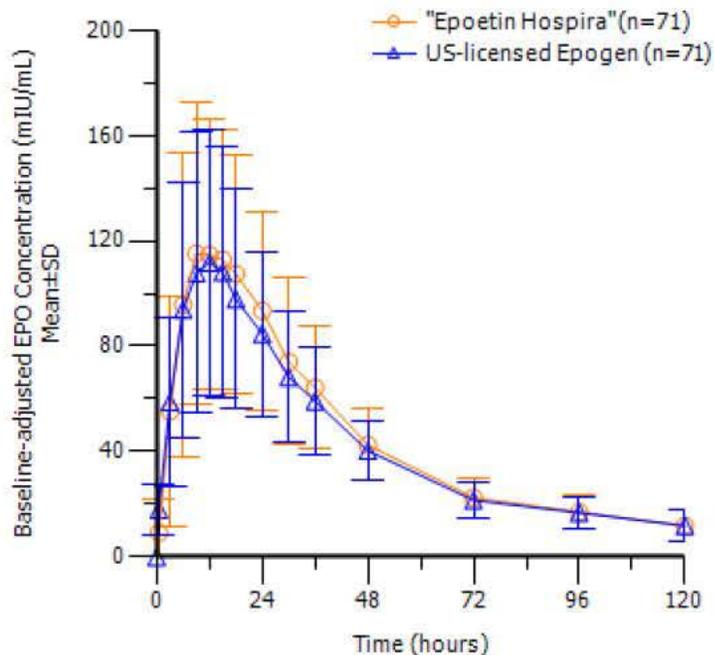
Table 8. Statistical analyses of pharmacokinetic parameters in Study EPOE-12-02

| PK Endpoints | Geometric Mean Ratio (90% CI) |
|---------------------------|--------------------------------------|
| AUC_{0-120h} (mIU·h/mL) | 1.06 (1.01, 1.11) |
| AUC_{0-INF} (mIU·h/mL) | 1.03 (0.97, 1.09) |
| C_{MAX} (mIU/mL) | 1.09 (1.01, 1.18) |

Source: FDA analysis of data from Hospira 351(k) BLA submission



Figure 10. Pharmacokinetic profiles following 100 U/kg subcutaneous single dose of “Epoetin Hospira” or US-licensed Epogen/Procrit in healthy subjects in Study EPOE-12-02.



Source: FDA analysis of data from Hospira 351(k) BLA submission

Pharmacodynamic Results (Reticulocyte Count)

In Study EPOE-12-02, the pairwise comparison of “Epoetin Hospira and US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PD similarity (90% CIs for the ratios of geometric mean of reticulocyte count (expressed as a percentage) AUEC_{0-456h}, and E_{MAX} within 80% to 125% range) as summarized in Table 9 and depicted in Figure 11.

These data establish the PD (reticulocyte count) similarity between “Epoetin Hospira” and US-licensed Epogen.

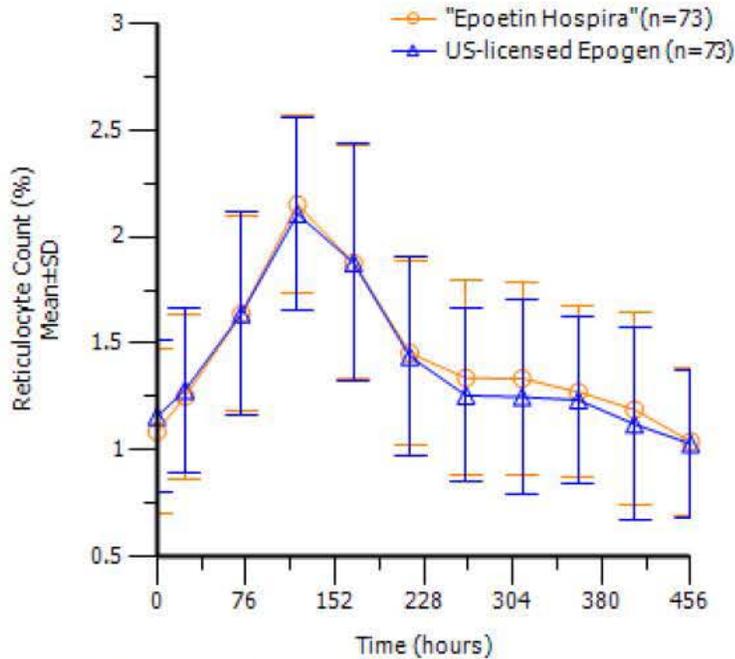
Table 9. Statistical analyses of pharmacodynamic parameters (reticulocyte count) in Study EPOE-12-02.

| PD Endpoints | Geometric Mean Ratio (90% CI) |
|------------------------------|-------------------------------|
| AUEC _{0-456h} (%·h) | 1.01 (0.98, 1.05) |
| E _{MAX} (%) | 1.02 (0.99, 1.05) |

Source: FDA analysis of data from Hospira 351(k) BLA submission



Figure 11. Pharmacodynamic profiles (reticulocyte count) following 100 U/kg subcutaneous single dose of “Epoetin Hospira” or US-licensed Epogen/Procrit in healthy subjects in Study EPOE-12-02.



Source: FDA analysis of data from Hospira 351(k) BLA submission

Study EPOE-14-01

Pharmacokinetic Results

In Study EPOE-14-01, the multiple-dose PK findings are considered supportive to the pivotal Study EPOE-12-02 (single-dose study) to assess PK similarity. PK was evaluated following the last dose on Day 26 through 48 hours post-dose. The pairwise comparison of “Epoetin Hospira” and US-licensed Epogen met the pre-specified acceptance criteria for PK similarity (90% CIs for the ratios of geometric mean of AUC_{0-T}, and C_{MAX}, within 80% to 125% range) as summarized in Table 10 and depicted in Figure 12.

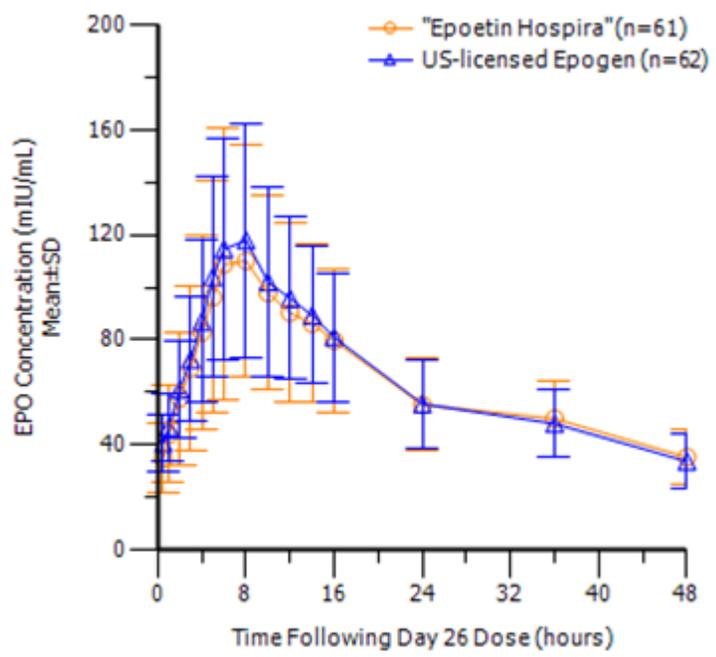
Table 10. Statistical analyses of pharmacokinetic parameters in Study EPOE-14-01

| PK Endpoints | Geometric Mean Ratio (90% CI) |
|---------------------------------|--------------------------------------|
| AUC _{0-48h} (mIU·h/mL) | 0.97 (0.90, 1.06) |
| C _{MAX} (mIU/mL) | 0.94 (0.84, 1.05) |

Source: FDA analysis of data from Hospira 351(k) BLA submission



Figure 12. Pharmacokinetic profiles following multiple doses of 100 U/kg subcutaneous three times per week for 4 weeks in healthy subjects in Study EPOE-14-01



Source: FDA analysis of data from Hospira 351(k) BLA submission

Pharmacodynamic Results (Hemoglobin Level)

In Study EPOE-14-01, the pairwise comparison of “Epoetin Hospira” and US-licensed Epogen/Procrit met the pre-specified acceptance criteria for PD similarity (90% CIs for the ratios of geometric mean of hemoglobin level $AUEC_{0-28d}$, and E_{MAX} within 80% to 125% range) as summarized in Table 11 and depicted in Figure 13.

These data establish the PD (hemoglobin level) similarity between “Epoetin Hospira and US-licensed Epogen/Procrit.

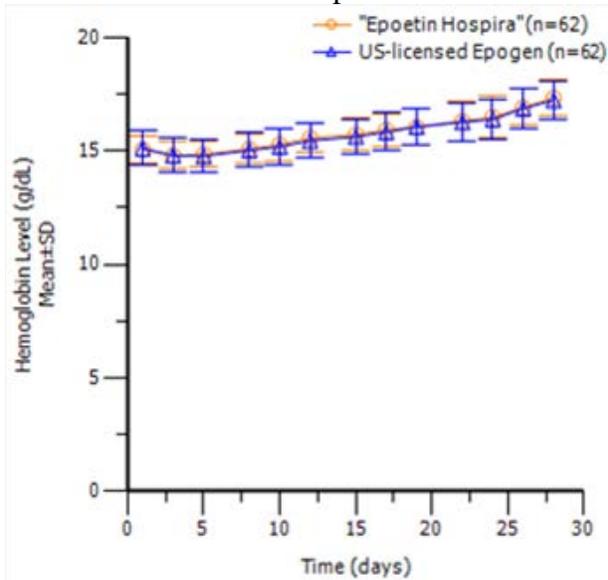
Table 11. Statistical analyses of pharmacodynamic parameters (hemoglobin level) in Study EPOE-14-01

| PD Endpoints | Geometric Mean Ratio (90% CI) |
|-------------------------|--------------------------------------|
| $AUEC_{0-28d}$ (g·h/dL) | 1.00 (0.99, 1.02) |
| E_{MAX} (g/dL) | 1.00 (0.99, 1.02) |

Source: FDA analysis of data from Hospira 351(k) BLA submission



Figure 13. Pharmacodynamic profiles (hemoglobin level) following multiple doses of 100 U/kg subcutaneous three times per week for 4 weeks in healthy subjects in Study EPOE-14-01

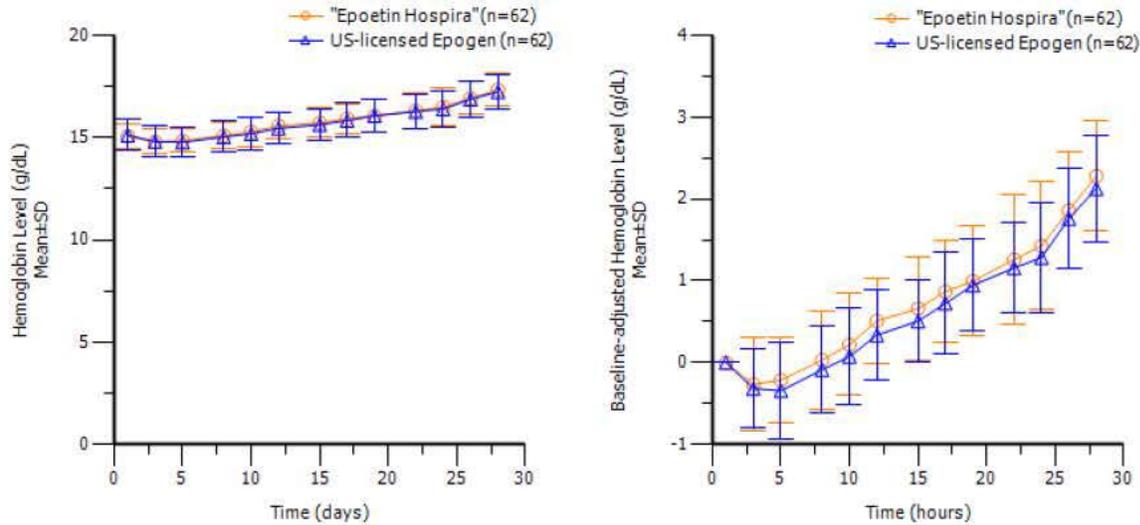


Source: FDA analysis of data from Hospira 351(k) BLA submission

A significant portion of the hemoglobin level AUEC is contributed by baseline hemoglobin levels, which may result in the overall profile to be insensitive to detect PD (hemoglobin level) differences between products. To explore this, FDA conducted de novo analysis of the observed hemoglobin profiles by calculating baseline-adjusted levels, as shown side-by-side with mean hemoglobin level profile (Figure 14). Further, FDA used a linear regression model on both hemoglobin level and baseline-adjusted hemoglobin level profiles to compare the effect of treatment arm and time on hemoglobin levels (Figure 15). The results of these analyses showed that following 100 U/kg SC TIW for 4 weeks dosing in healthy subjects, hemoglobin level increases by approximately 0.086 g/dL each day. The interaction effect was not significant between day and arm ($p=0.760$ for hemoglobin level profile, $p=0.697$ for baseline-adjusted hemoglobin level profile). These findings provide further evidence to support similarity of PD (hemoglobin level) response between “Epoetin Hospira” and US-licensed Epogen/Procrit.

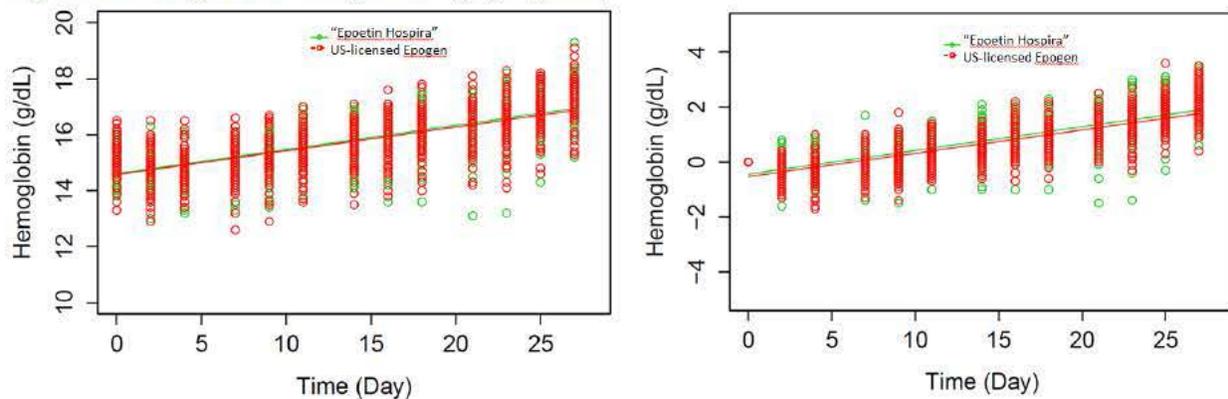


Figure 14. Hemoglobin vs. time profile using (left) hemoglobin level and (right) baseline-adjusted hemoglobin level



Source: FDA analysis of data from Hospira 351(k) BLA submission

Figure 15. Linear regression model of hemoglobin level profiles (left panel) and baseline-adjusted hemoglobin level profiles (right panel)



Source: FDA analysis of data from Hospira 351(k) BLA submission

Clinical Pharmacology Summary

Overall, the submitted clinical pharmacology studies adequately demonstrated similarity of PK and PD (reticulocyte count and hemoglobin level) between “Epoetin Hospira” and US-licensed Epogen/Procrit.

Studies EPOE-12-02 and EPOE-14-01, conducted in healthy subjects using a SC administration route, are considered sufficiently sensitive to detect clinically significant differences in PK and PD (reticulocyte count and hemoglobin level) among the products. Single-dose PK and PD (reticulocyte count) and multiple-dose PD (hemoglobin level) similarity pre-specified margins were met. The demonstration of similar PK and PD (reticulocyte count and hemoglobin level)



exposure supports a demonstration of no clinically meaningful differences between “Epoetin Hospira” and US-licensed Epogen/Procrit.

7 Efficacy and Safety

Executive Summary

Hospira submitted two clinical studies that evaluated efficacy and safety endpoints in support of licensure of “Epoetin Hospira”. Both studies were randomized, double-blinded, parallel group studies that enrolled patients with chronic kidney disease on hemodialysis and receiving epoetin maintenance treatment with co-primary endpoints of difference between arms in mean weekly hemoglobin and mean weekly dose. One study (EPOE-10-13) used subcutaneous epoetin, and the other study (EPOE-10-01) used intravenous epoetin. The FDA review of the data from both studies supports the Applicant’s conclusion that there are no clinically meaningful differences in efficacy and safety between “Epoetin Hospira” and US-licensed Epogen/Procrit.

Discussion

Study Description. The Applicant submitted the results from two clinical studies that evaluated efficacy and safety endpoints: EPOE-10-13 and EPOE-10-01. Both were randomized, double-blinded, parallel group studies that enrolled patients with chronic kidney disease on hemodialysis used to compare "Epoetin Hospira" to US-licensed Epogen/Procrit administered subcutaneously (EPOE-10-13) or intravenously (EPOE-10-01). Both studies had an optional long-term safety study under separate protocols for an additional 48 weeks of treatment. A summary of the two clinical studies is provided in Table 12.

Table 12. Summary of clinical studies

| Study ID | Design | Route | Number in ITT | Subjects | Dose | Schedule | Primary Endpoint |
|------------|----------|-------|---------------|-------------------|----------|------------------|------------------------------------|
| EPOE-10-13 | Parallel | SC | 246 | Patients with CKD | Variable | 1-3 times / week | Mean weekly Hb Mean weekly dose |
| EPOE-10-01 | Parallel | IV | 612 | Patients with CKD | Variable | 1-3 times / week | Mean weekly Hb Mean weekly dose |

Abbreviations: SC = subcutaneous, IV = intravenous, CKD = chronic kidney disease, Hb = hemoglobin

Source: Summary based on information from Hospira 351(k) BLA submission

EPOE-10-13 was a randomized, double-blind, parallel group phase 3 study in which patients with CKD requiring hemodialysis and receiving EPO maintenance treatment. In the titration period of the study, patients who previously received intravenous US-licensed Epogen/Procrit were randomized to subcutaneous "Epoetin Hospira" or US-licensed Epogen/Procrit for 12 to 18 weeks to achieve 4 weeks of stable dosing. Patients who had been on subcutaneous US-licensed Epogen/Procrit were randomized directly into the maintenance period. In the maintenance period, patients were randomized to either "Epoetin Hospira" or US-licensed Epogen/Procrit for 16 weeks.



EPOE-10-01 enrolled the same patient population with only patients on prior IV US-licensed Epogen/Procrit included therefore obviating the need for a titration period. In the maintenance period, patients were randomized to intravenous "Epoetin Hospira" or US-licensed Epogen/Procrit for 24 weeks.

In both studies, patient demographics and baseline disease characteristics were evenly distributed between arms with only minor imbalances. Subject disposition was balanced between treatment arms.

GCP compliance issues. The Applicant disclosed that multiple sites in both studies were Good Clinical Practice (GCP) non-compliant. Examples of GCP issues from the sites excluded from the analyses include: lack of principal investigator oversight, protocol deviations (involving eligibility criteria, dosing errors, drug storage), inconsistent documentation, discrepancies in informed consent documentation, and safety under-reporting. Across the two studies, 121 unique sites enrolled patients (68 sites in EPOE-10-13 and 95 sites in EPOE-10-01). During the conduct of the study, seven sites were closed, and after a post-study audit by the Applicant, two additional sites were identified as having GCP compliance issues. In study EPOE-10-13, 3 sites closed which included 10% (53/556) of enrolled subjects and 8% (20/246) of the subjects in the intent-to-treat population. In study EPOE-10-01, seven sites were closed during the study, and two additional non-GCP compliant sites identified which represented 14% (140/1017) of subjects enrolled and 11% (65/612) of subjects in the intent-to-treat (ITT) population. The three sites closed in EPOE-10-13 also participated in EPOE-10-01. The FDA conducted sensitivity analyses for both efficacy and safety endpoints excluding the GCP non-compliant sites to confirm the integrity of the initial analysis.

Efficacy Analysis

Study Design. The primary endpoints for the comparative clinical studies were:

- Mean weekly hemoglobin (Hb) level during the last 4 weeks of the double-blind Treatment Period.
- Mean weekly dosage per kg body weight during the last 4 weeks of the double-blind Treatment Period.

Sample size determination. Parameter estimates used to determine the sample size are described in Table 13.



Table 13. Sample size calculation

| | Parameter | Power | Equivalence Margin | SD | Assumed % drop-out | Planned N |
|-----------------|------------------|--------------|---------------------------|-----------|---------------------------|------------------|
| EPOE-10-13 (SC) | Hb (g/dL) | 90% | ± 0.5 | 0.94 | 35% | 288 |
| | Dose (U/kg/week) | | ± 45 | 78 | | |
| EPOE-10-01 (IV) | Hb (g/dL) | 90% | ± 0.5 | 1.37 | 30% | 564 |
| | Dose (U/kg/week) | | ± 45 | 118.11 | | |

Source: FDA analysis of data from Hospira 351(k) BLA submission

Based on the parameters specified in the table, a total of 288 and 564 subjects were planned for EPOE-10-13 and EPOE-10-01, respectively.

Selection of Hemoglobin Equivalence Margin of ± 0.5 g/dL. 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. [Berns et al. 2003; Eschbach et al. 1987; Krivoshiev et al. 2008; Krivoshiev et al. 2010; Lacson et al. 2003; Wizemann et al. 2008]

Selection of Dose Acceptance Margin of ± 45 U/kg/Week. The dose acceptance margin of ± 45 U/kg/week was informed by the following:

- The establishment of the no-effect dose of 45 U/kg/week from multiple sources [same references as in selection of hemoglobin equivalence margin];
- The demonstration that doses of 150 U/kg/week or higher were needed to provide a consistent dose-dependent increase in Hb;
- The recommended increase or decrease of ± 37.5 to ± 75 U/kg/week of the starting dose for patients who required a dose modification per the Epogen/Procrit reference product labeling;

Analysis Populations. The following are the definitions for the analysis populations.

- Intent-to-treat (ITT) Population: all subjects who were randomized into the Maintenance Period.
- GCP-population: a subset of the ITT population without the subjects from the closed sites.
- Per Protocol (PP) Population: a subset of the ITT population who met the following criteria:
 - Had at least 4 weeks of treatment with study drug in the Maintenance Period
 - Had at least 4 weeks of Hb data collected while on study drug during the Maintenance Period
 - Had at least 4 weeks of study drug administration data collected while on study drug during the Maintenance Period
 - Had no important protocol deviation



- Had no use of other ESAs during the last 4 weeks of study drug administration
- Had received no packed red blood cells or whole blood transfusions during study conduct.

Statistical Analysis for Primary Endpoints. The primary objectives of the studies were to support a demonstration that there are no clinically meaningful differences between “Epoetin Hospira” compared to US-licensed Epogen/Procrit based on the two co-primary endpoints in both subcutaneous (SC) and intravenous (IV) studies, EPOE-10-13 and EPOE-10-01, respectively.

The 2-sided 90% CIs for the difference in mean between “Epoetin Hospira” and US-licensed Epogen/Procrit of two primary efficacy measurements were calculated with an analysis of covariance (ANCOVA) and compared with pre-defined therapeutic acceptance ranges. The ANCOVA model contains effects for treatment and baseline values (either baseline Hb or baseline dose).

In the Applicant’s submitted analysis, 95% confidence intervals were used to test for clinically meaningful differences. This is equivalent to testing at the 2.5% type I error level in the similarity test. For biosimilar comparisons, FDA accepts a type I error level of 5% which can be achieved using 90% confidence intervals.

Multiple Comparison/Multiplicity. A hierarchical test strategy was used for the testing of the co-primary endpoints in the following order: difference in the mean Hb level, difference in mean weekly dose in the last 4 weeks of Maintenance Period.

Missing Data Handling Strategies. There is no missing data imputation scheme for the primary analysis.

Efficacy Analysis Results. For the results presented below, FDA used the GCP-population as the primary analysis population for the co-primary endpoints.

EPOE-10-13 (SC). The mean weekly Hb and the mean weekly dose of epoetin per kg of body weight during the last 4 weeks of the treatment period for the “Epoetin Hospira” and the US-licensed Epogen/Procrit treatment groups, as well as the difference in those two co-primary endpoints between the groups are shown in Table 14 for both the original ITT population and the GCP-population without the subjects from the closed sites.

For the ITT population, the least square (LS) mean, which is the mean adjusted for baseline values in the ANCOVA model, for the difference between the “Epoetin Hospira” group and the US-licensed Epogen/Procrit group in weekly Hb during the last 4 weeks of the treatment period was 0.04g/dL, with a 90% CI of -0.13 to 0.21 g/dL. For the GCP-population, the LS mean for the difference between the “Epoetin Hospira” group and the US-licensed Epogen/Procrit group in weekly Hb during the last 4 weeks of the treatment period was 0.04g/dL, with a 90% CI of -0.13 to 0.22 g/dL. The 90% CIs are contained within the pre-specified acceptance limits of -0.5 to 0.5 g/dL for both analysis populations. No clinically meaningful difference existed in Hb



levels between the two treatment groups during the last 4 weeks of the treatment period in either analysis population.

By the hierarchical testing strategy, this conclusion allowed further assessment of the two treatment groups for epoetin dose. For the original ITT population, the LS mean for the difference between the two treatment arms during the last 4 weeks of the treatment period was -2.34 U/kg/week, with the 90% CI of -12.54 to 7.85 U/kg/week. For the GCP-population, the LS mean for the difference between the two treatment arms during the last 4 weeks of the treatment period was 0.76 U/kg/week, with a 90% CI of -8.86 to 10.38 U/kg/week. The 90% CI for both populations are contained within the pre-specified acceptance limits of -45 to 45 U/kg/week. No clinically meaningful difference existed in weekly dose per kg body weight between the two treatment groups during the last 4 weeks of the treatment period in either analysis population.

Table 14. Results for the difference in mean weekly hemoglobin and mean weekly dose in the last 4 weeks of double blind treatment period for Study EPOE-10-13, subcutaneous treatment

| EPOE-10-13 (SC) | Original ITT | | Closed Sites Excluded | |
|---|---------------------------|----------------------------|---------------------------|----------------------------|
| | “Epoetin Hospira” (n=124) | US- Epogen/Procrit (n=122) | “Epoetin Hospira” (n=112) | US- Epogen/Procrit (n=114) |
| Hemoglobin (g/dL) | | | | |
| LS Means (SE) | 10.2 (0.07) | 10.1 (0.07) | 10.2 (0.08) | 10.1 (0.08) |
| LS Difference | 0.04 (0.104) | | 0.04 (0.108) | |
| 90% CI for Diff. | (-0.13, 0.21) | | (-0.13, 0.22) | |
| Equivalence Margin | (-0.5, 0.5) | | (-0.5, 0.5) | |
| Dose per Kg Body Weight(U/kg/week) | | | | |
| LS Means (SE) | 79.6 (4.36) | 81.9 (4.37) | 74.8 (4.15) | 74.1 (4.09) |
| LS Difference | -2.34 (6.175) | | 0.76 (5.824) | |
| 90% CI for Diff. | (-12.54, 7.85) | | (-8.86, 10.38) | |
| Equivalence Margin | (-45, 45) | | (-45, 45) | |

Source: FDA analysis of data from Hospira 351(k) BLA submission

EPOE-10-01 (IV): The mean weekly Hb and the mean weekly dosage of epoetin per kg of body weight during the last 4 weeks of the treatment period for the “Epoetin Hospira” and the US-licensed Epogen/Procrit treatment groups, as well as the difference in those two-co-primary endpoints between the groups are shown in Table 15 for both the original ITT population and the GCP-population. The results from the co-primary endpoints agree with the primary analysis results from the SC study described in the previous section.

For the original ITT population, the LS mean for the difference between the “Epoetin Hospira” group and the US-licensed Epogen/Procrit group in weekly Hb during the last 4 weeks of the treatment period was -0.12g/dL, with a 90% CI of -0.22 to -0.01 g/dL. For the GCP-population, the LS mean for the difference between the “Epoetin Hospira” group and the US-licensed Epogen/Procrit group in weekly Hb during the last 4 weeks of the treatment period was -0.11g/dL, with a 90% CI of -0.22 to 0.01 g/dL. The 90% CIs for both population are contained

within the pre-specified acceptance limits of -0.5 to 0.5 g/dL. No clinically meaningful difference existed in Hb levels between the two treatment groups during the last 4 weeks of the treatment period in either analysis population.

By the hierarchical testing strategy, this conclusion allowed further assessment of the two treatment groups for epoetin dose. For the original ITT population, the LS mean for the difference between the two treatment arms during the last 4 weeks of the treatment period was 0.37 U/kg/week, with a 90% CI of -8.67 to 9.40 U/kg/week. For the GCP-population, the LS mean for the difference between the two treatment arms during the last 4 weeks of the treatment period was 0.80 U/kg/week, with a 90% CI of -8.32 to 9.92 U/kg/week. The 90% CI are contained within the pre-specified acceptance limits of -45 to 45 U/kg/week for both populations. No clinically meaningful difference existed in weekly dose per kg body weight between the two treatment groups during the last 4 weeks of the treatment period in either analysis population.

Table 15. Results for the difference in mean weekly hemoglobin and mean weekly dose in the last 4 weeks of double blind treatment period for Study EPOE-10-01, intravenous treatment

| EPOE-10-01 (IV) | Original ITT | | Closed Sites Excluded | |
|---|---------------------------|----------------------------|---------------------------|----------------------------|
| | “Epoetin Hospira” (n=306) | US- Epogen/Procrit (n=306) | “Epoetin Hospira” (n=268) | US- Epogen/Procrit (n=279) |
| Hemoglobin (g/dL) | | | | |
| LS Means (SE) | 10.2 (0.05) | 10.3 (0.05) | 10.2 (0.05) | 10.3 (0.05) |
| LS Difference | -0.12 (0.066) | | -0.11 (0.070) | |
| 90% CI for Diff. | (-0.22, -0.01) | | (-0.22, 0.01) | |
| Equivalence Margin | (-0.5, 0.5) | | (-0.5, 0.5) | |
| Dose per Kg Body Weight(U/kg/week) | | | | |
| LS Means (SE) | 90.2 (3.87) | 89.8 (3.88) | 87.9 (4.02) | 87.6 (3.95) |
| LS Difference | 0.37 (5.483) | | 0.29 (5.637) | |
| 90% CI for Diff. | (-8.67, 9.40) | | (-9.00, 9.58) | |
| Equivalence Margin | (-45, 45) | | (-45, 45) | |

Source: FDA analysis of data from Hospira 351(k) BLA submission

For both EPOE-10-13 and EPOE-10-01, sensitivity analyses using the different analysis populations (per protocol, GCP population, and imputed missing) showed results that were consistent with the results for the original ITT population. Subgroup analyses for sex, age, and race also showed results that were consistent with the results of the primary analysis.

Safety Analysis

Methods. The Safety Populations in the randomized phase 3 studies consisted of all subjects who received at least one dose of study drug. The safety assessments presented herein are during the randomized maintenance period of both studies.



Results. An overview of the frequency of treatment-emergent adverse events (TEAE) is shown in Table 16 for study EPOE-10-13 and Table 17 for EPOE-10-01, for subcutaneous and intravenous treatment, respectively. No clinically meaningful differences in the frequency of TEAEs were seen in either study.

Table 16. Frequency of treatment-emergent adverse events in Study EPOE-10-13, subcutaneous treatment

| | Original Analysis | | Closed Sites Excluded | |
|---|-----------------------------|-----------------------------|---------------------------|---------------------------|
| | “Epoetin Hospira” (N = 122) | US-Epogen/Procrit (N = 122) | “Epoetin Hospira” (N=110) | US-Epogen/Procrit (N=114) |
| Subjects Reporting at Least One TEAE | 85 (70) | 86 (71) | 79 (72) | 79 (69) |
| Subjects Reporting at Least One Serious TEAE | 23 (19) | 33 (27) | 19 (17) | 29 (25) |
| Subjects Discontinuing Study Drug due to a TEAE | 4 (3) | 4 (3) | 4 (4) | 4 (4) |
| Subjects Reporting an TEAE Resulting in Death | 3 (3) | 2 (2) | 3 (3) | 2 (2) |

Source: FDA analysis of data from Hospira 351(k) BLA submission

Table 17. Frequency of treatment-emergent adverse events in Study EPOE-10-01, intravenous treatment

| | Original Analysis | | Closed Sites Excluded | |
|---|-----------------------------|-----------------------------|---------------------------|---------------------------|
| | “Epoetin Hospira” (N = 301) | US-Epogen/Procrit (N = 304) | “Epoetin Hospira” (N=264) | US-Epogen/Procrit (N=277) |
| Subjects Reporting at Least One TEAE | 232 (77) | 229 (75) | 207 (78) | 210 (76) |
| Subjects Reporting at Least One Serious TEAE | 75 (25) | 82 (27) | 64 (24) | 77 (28) |
| Subjects Discontinuing Study Drug due to a TEAE | 9 (3) | 11 (4) | 9 (3) | 11 (4) |
| Subjects Reporting an TEAE Resulting in Death | 5 (2) | 6 (2) | 3 (1) | 6 (2) |

Source: FDA analysis of data from Hospira 351(k) BLA submission

The common adverse events were not considerably different between "Epoetin Hospira" and US-licensed Epogen/Procrit. Common AEs seen in $\geq 5\%$ of patients in any group are shown in Table 18 for EPOE-01-13 and in Table 19 for EPOE-10-01, including the original analysis and after removal of sites closed for GCP issues.



Table 18. Frequency of common adverse events ($\geq 5\%$ in any group) in Study EPOE-10-13, subcutaneous treatment

| | Original Analysis | | Closed Sites Excluded | |
|---|-----------------------------|-------------------------------|---------------------------|-----------------------------|
| | “Epoetin Hospira” (N = 122) | US- Epogen/ Procrit (N = 122) | “Epoetin Hospira” (N=110) | US- Epogen/ Procrit (N=114) |
| Nausea | 10 (8) | 8 (7) | 10 (9) | 7 (6) |
| Fall | 8 (7) | 3 (2) | 7 (6) | 3 (3) |
| Pyrexia | 8 (7) | 4 (3) | 7 (6) | 3 (3) |
| Arteriovenous fistula site complication | 6 (5) | 4 (3) | 6 (6) | 4 (4) |
| Headache | 6 (5) | 3 (2) | 5 (5) | 2 (2) |
| Pain in extremity | 6 (5) | 5 (4) | 5 (5) | 5 (4) |
| Dizziness | 3 (2) | 9 (7) | 3 (3) | 9 (8) |
| Injection site pain | 3 (2) | 8 (7) | 2 (2) | 6 (5) |
| Vomiting | 4 (3) | 6 (5) | 2 (2) | 6 (5) |
| Hyperkalemia | 3 (2) | 6 (5) | 3 (3) | 5 (4) |
| Hypoglycemia | 1 (1) | 6 (5) | 1 (1) | 6 (5) |
| Arthralgia | 5 (4) | 4 (3) | 5 (5) | 4 (4) |
| Urinary tract infection | 5 (4) | 4 (3) | 5 (5) | 4 (4) |

Source: FDA analysis of data from Hospira 351(k) BLA submission



Table 19. Frequency of common adverse events ($\geq 5\%$ in any group) in Study EPOE-10-01, intravenous treatment

| | Original Analysis | | Closed Sites Excluded | |
|---|-----------------------------|-------------------------------|---------------------------|-----------------------------|
| | “Epoetin Hospira” (N = 301) | US- Epogen/ Procrit (N = 304) | “Epoetin Hospira” (N=264) | US- Epogen/ Procrit (N=277) |
| Nausea | 30 (10) | 25 (8) | 27 (10) | 24 (9) |
| Vomiting | 28 (9) | 15 (5) | 26 (10) | 14 (5) |
| Muscle spasms | 27 (9) | 24 (8) | 27 (10) | 19 (7) |
| Arteriovenous fistula site complication | 26 (9) | 25 (8) | 23 (9) | 24 (9) |
| Headache | 23 (8) | 16 (5) | 23 (9) | 14 (5) |
| Dyspnea | 22 (7) | 21 (7) | 22 (8) | 20 (7) |
| Diarrhea | 21 (7) | 27 (9) | 18 (7) | 27 (10) |
| Dizziness | 20 (7) | 15 (5) | 17 (6) | 14 (5) |
| Hypertension | 19 (6) | 12 (4) | 17 (6) | 11 (4) |
| Cough | 16 (5) | 22 (7) | 16 (6) | 22 (8) |
| Hyperkalemia | 14 (5) | 12 (4) | 13 (5) | 12 (4) |
| Hypotension | 14 (5) | 23 (8) | 9 (3) | 19 (7) |
| Pain in extremity | 10 (3) | 17 (6) | 9 (3) | 14 (5) |
| Non-cardiac chest pain | 7 (2) | 17 (6) | 6 (2) | 14 (5) |
| Back pain | 12 (4) | 16 (5) | 12 (5) | 15 (5) |
| Arthralgia | 13 (4) | 12 (4) | 13 (5) | 11 (4) |
| Fall | 13 (4) | 13 (4) | 13 (5) | 12 (4) |
| Anemia | 8 (3) | 13 (4) | 8 (3) | 13 (5) |
| Constipation | 7 (2) | 13 (4) | 6 (2) | 13 (5) |

Source: FDA analysis of data from Hospira 351(k) BLA submission

The frequency of TEAE, serious events, and events leading to discontinuation of study drug or death was not different between the treatment arms. Major events of interest which are listed as Warnings and Precautions in the prescribing information for US-licensed Epogen/Procrit include myocardial infarction, cerebrovascular events, and thromboembolism. Events in these categories occurred in both studies with no imbalances between treatment arms, see Table 20 and Table 21 for the frequency of these events in studies EPOE-10-13 and EPOE-10-01, respectively. There were no cases of pure red cell aplasia (PRCA) in the randomized studies. A sensitivity analysis excluding non-GCP compliant sites did not change the overall results.



Table 20. Adverse events of special interest in Study EPOE-01-13, subcutaneous treatment.

| | Original Analysis | | Closed Sites Excluded | |
|------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------|
| | “Epoetin Hospira” (N = 122) | US-Epogen/Procrit (N = 122) | “Epoetin Hospira” (N=110) | US-Epogen/Procrit (N=114) |
| Myocardial infarction | 0 | 1 (1) | 0 | 1 (1) |
| Cerebrovascular events | 0 | 2 (2) | 0 | 2 (2) |
| Thromboembolic events | 4 (3) | 8 (7) | 4 (4) | 7 (6) |

Source: FDA analysis of data from Hospira 351(k) BLA submission

Table 21. Adverse events of special interest in Study EPOE-01-01, intravenous treatment.

| | Original Analysis | | Closed Sites Excluded | |
|------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------|
| | “Epoetin Hospira” (N = 301) | US-Epogen/Procrit (N = 304) | “Epoetin Hospira” (N=264) | US-Epogen/Procrit (N=277) |
| Myocardial infarction | 4 (1) | 2 (1) | 2 (1) | 2 (1) |
| Cerebrovascular events | 4 (1) | 4 (1) | 3 (1) | 3 (1) |
| Thromboembolic events | 28 (9) | 18 (6) | 24 (9) | 17 (6) |

Source: FDA analysis of data from Hospira 351(k) BLA submission

Conclusion

Efficacy. In summary, the 90% confidence intervals for the difference between “Epoetin Hospira” and US-licensed Epogen/Procrit in both EPOE-10-13 (SC) and EPOE-10-01 (IV) studies are within the equivalence margins. Results from sensitivity analyses and subgroup analyses were consistent and agree with the primary analysis result. Results obtained the GCP-population are similar to the results obtained from the original ITT population.

Safety. The safety results of studies EPOE-10-13 and EPOE-10-01 did not show evidence of any clinically meaningful differences between "Epoetin Hospira" and US-licensed Epogen/Procrit.

8 Risk Evaluation and Mitigation Strategy (REMS)

A REMS is a required risk management plan that uses risk minimization strategies beyond professional labeling that is designed to ensure that the benefits of certain prescription drugs outweigh their risks. Under the Food and Drug Administration Amendments Act of 2007 (FDAAA), the FDA has the authority to require a manufacturer to develop and implement a REMS to manage a known or potential serious risk associated with a drug and enable patients to have continued access to the drug. Additional information and background on REMS is on the [FDA Basics Webinar: A Brief Overview of REMS webpage](#) on this topic.



In 2010, the FDA approved a REMS program for Aranesp and Epogen/Procrit to ensure their benefits for use as a treatment alternative to RBC transfusion for anemia associated with myelosuppressive chemotherapy, outweigh their risks of shortened overall survival and/or increased risk of tumor progression or recurrence in patients with cancer. The REMS submitted by Hospira is similar to the Epogen/Procrit REMS Program.

On April 13, 2017 the FDA stated that a REMS is no longer necessary to ensure that the benefits of Epogen/Procrit and Aranesp outweigh the risks of shortened overall survival and/or increased risk of tumor progression or recurrence, for the treatment of anemia associated with myelosuppressive chemotherapy. While the REMS for the ESAs has been removed, this does not mean the risks have disappeared, therefore the prescribing information and Medication Guide continues to note an increased risk of tumor progression or recurrence, as well as death, myocardial infarction, stroke, venous thromboembolism, and thrombosis of vascular access.

The risks of approved ESAs, including any biosimilar product(s) (if approved), will continue to be communicated through the product prescribing information and the Medication Guide. The appropriate use of ESAs is supported by the Centers for Medicare & Medicaid Services National Coverage Determination (CMS NCD), the American Society of Clinical Oncology (ASCO) and American Society of Hematology (ASH) clinical guidelines which are evidence-based guidelines intended to provide a basis for the standard of care in clinical oncology.

9 Extrapolation Across Indications

The Applicant seeks licensure for all indications for which US-licensed Epogen/Procrit is licensed (listed in Introduction section above). The “Epoetin Hospira” clinical program, however, provides clinical efficacy and safety data from a clinical program in patients with chronic kidney disease on hemodialysis.

FDA has determined that it may be appropriate for a biosimilar product to be licensed for one or more conditions of use (e.g., indications) for which the reference product is licensed, based on data from a clinical study(ies) performed in another condition of use. This concept is known as extrapolation. As described in the Guidance for Industry: “Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009”, if a biological product meets the statutory requirements for licensure as a biosimilar product under section 351(k) of the PHS Act based on, among other things, data derived from a clinical study or studies sufficient to demonstrate safety, purity, and potency in an appropriate condition of use, the potential exists for that product to be licensed for one or more additional conditions of use for which the reference product is licensed [FDA Guidance: Biosimilars Questions and Answers, 2015]. The Applicant needs to provide sufficient scientific justification for extrapolation, which should address, for example, the following issues for the tested and extrapolated conditions of use:

- The mechanism(s) of action (MOA), if known or can reasonably be determined, in each condition of use for which licensure is sought,



- The pharmacokinetics (PK) and bio-distribution of the product in different patient populations,
- The immunogenicity of the product in different patient populations,
- Differences in expected toxicities in each condition of use and patient population,
- 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.

As a scientific matter, the FDA has determined that differences between conditions of use with respect to the factors addressed in a scientific justification for extrapolation do not necessarily preclude extrapolation.

The scientific justification for extrapolation of data to support a demonstration of biosimilarity in the indications for which the Applicant is seeking licensure include:

- The primary mode of action (MOA) of EPO is the same as endogenous erythropoietin. EPO binds to the erythropoietin receptor on specific erythrocyte precursor cells, which causes a conformational change in the receptor that brings its intracellular domains into close apposition enabling cross phosphorylation via the binding of JAK2 kinase, the initiation of the signal transduction cascade, and induction of erythropoiesis. This MOA is independent of the underlying cause of anemia. [Elliot et al. 2008]
- Demonstration that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit based on extensive analytical characterization data
- Similar pharmacokinetics/pharmacodynamics (PK/PD) was demonstrated between "Epoetin Hospira" and US-licensed Epogen/Procrit in healthy subjects, and a similar efficacy was demonstrated in patients with CKD on hemodialysis. A similar PK/PD profile would be expected between "Epoetin Hospira" and US-licensed Epogen/Procrit across the other indications for use.
- In the "Epoetin Hospira" clinical program, the frequency of anti-drug antibody formation was low and there were no notable differences between "Epoetin Hospira" and US-licensed Epogen/Procrit in both healthy male subjects and patients with CKD on hemodialysis. Accordingly, similar immunogenicity would be expected between "Epoetin Hospira" and US-licensed Epogen/Procrit in other indications of use.
- Similar clinical safety and efficacy profile was demonstrated between "Epoetin Hospira" and US-licensed Epogen/Procrit in patients with CKD on hemodialysis. As analytical and PK similarity was demonstrated between "Epoetin Hospira" and US-licensed Epogen/Procrit, a similar safety and efficacy profile would be expected in other indications for use.

In aggregate, the evidence indicates that the extrapolation of biosimilarity to the indications for which Hospira is seeking licensure is scientifically justified.



10 Conclusion

The totality of analytical data support the determination that “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit notwithstanding minor differences in clinically inactive components. The analytical similarity assessment was conducted using multiple orthogonal physicochemical and functional methods. The results support a demonstration that “Epoetin Hospira” is highly similar to US-Epogen/Procrit based on analyses of primary and high order structure, glycosylation, biological activity, product related species, and stability profiles, including degradation products and degradation pathways.

The pharmacodynamics, pharmacokinetics, and toxicity of “Epoetin Hospira” was compared head-to-head with US-licensed Epogen/Procrit via the subcutaneous route in Sprague-Dawley rats and intravenous route in Beagle dogs at doses with a frequency and duration of dosing consistent with the currently approved labeling of the US-licensed Epogen/Procrit reference product. After review of the submitted studies, from a pharmacology/toxicology perspective, there are residual uncertainties as to the similarity of “Epoetin Hospira” to US-licensed Epogen/Procrit based on the differences in pharmacodynamic activity observed following subcutaneous administration and plasma exposures following subcutaneous or intravenous administration. The residual uncertainties were addressed in the clinical pharmacology and clinical efficacy and safety data.

Anti-drug antibodies (including neutralizing antibodies) were measured using appropriately validated assays in three parallel arm clinical studies comparing “Epoetin Hospira” to US-licensed Epogen/Procrit: EPOE-10-01, EPOE-10-13 in patients with chronic kidney disease, and EPOE-14-01 in healthy volunteers. The data indicate that there is no clinically meaningful difference in immunogenicity risk for “Epoetin Hospira” when compared to US-licensed Epogen/Procrit.

The results of the pharmacokinetic and pharmacodynamic similarity studies support Hospira’s contention that there are no clinically meaningful differences in the effectiveness of “Epoetin Hospira” and US-licensed Epogen/Procrit for all of the indications for which US-licensed Epogen/Procrit is approved.

The results of the clinical development program indicate that Hospira’s data support a determination of no clinically meaningful differences between “Epoetin Hospira” and US-licensed Epogen/Procrit in terms of safety and efficacy in the indication studied. Specifically, the 90% confidence intervals for the difference between “Epoetin Hospira” and US-licensed Epogen/Procrit in both EPOE-10-13 and EPOE-10-01 studies are within the equivalence margins. Results from sensitivity analyses and subgroup analyses were consistent and agree with the primary analysis result. The safety analyses in both studies, which were randomized comparisons of “Epoetin Hospira” and US-licensed Epogen/Procrit in patients with chronic kidney disease on hemodialysis, did not show any new safety signals.



In considering the totality of the evidence, the data submitted by the Applicant show that "Epoetin Hospira" is highly similar to US-licensed Epogen/Procrit, notwithstanding minor differences in clinically inactive components, and support a demonstration that there are no clinically meaningful differences between "Epoetin Hospira" and US-licensed Epogen/Procrit in terms of the safety, purity, and potency of the product.

FDA requests discussion at the Oncologic Drugs Advisory Committee to obtain feedback and insights whether the totality of evidence presented support licensure of “Epoetin Hospira” as a biosimilar to US-licensed Epogen/Procrit. This determination requires the following criteria to be met:

- “Epoetin Hospira” is highly similar to US-licensed Epogen/Procrit, notwithstanding minor differences in clinically inactive components, and
- There are no clinically meaningful differences between “Epoetin Hospira” and US-licensed Epogen/Procrit.

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