BACKGROUND INFORMATION

FOR

ONCOLOGIC DRUGS ADVISORY COMMITTEE

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Safety of Erythropoiesis-Stimulating Agents (ESAs) in Oncology

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List of Abbreviations

AHRQ	Agency for Healthcare Research and Quality
BLA	Biologic License Application
BLS	Biologic License Supplement
CIA	chemotherapy-induced anemia
CLL	chronic lymphocytic leukemia
CV/TE	Cardiovascular and thromboembolic events
DAHANCA	Danish Head and Neck Cancer Study Group
DMC	data monitoring committee
DSMB	data safety monitoring board
ECOG	Eastern Cooperative Oncology Group
EOTP	end of treatment phase
EPO	erythropoietin
EpoR	erythropoietin receptor
ESA(s)	erythropoiesis-stimulating agent(s)
GELA	Group d'Etude des Lymphomes de l'Adulte
Hgb	hemoglobin
HR	hazards ratio
J&JPRD	Johnson & Johnson Pharmaceutical Research and Development
NESP	novel erythropoiesis stimulating protein, darbepoetin alfa
NHL	Non-Hodgkin's lymphoma
ODAC	Oncologic Drugs Advisory Committee
OR	odds ratio
QW	once weekly
Q2W	once every 2 weeks
Q3W	once every 3 weeks
RBC	red blood cell
rHuEPO	recombinant human erythropoietin
RR	risk ratio
SC	subcutaneous
SE	standard error
TE	thromboembolic event
TVE	thrombotic vascular event

1. Executive Summary

Erythropoiesis-stimulating agents (ESAs) engage the same receptor and trigger the same physiological response as endogenous erythropoietin (EPO). Darbepoetin alfa and epoetin alfa are ESAs licensed for the treatment of anemia in patients with nonmyeloid malignancy receiving chemotherapy. In this patient population, ESAs provide the only alternative to blood transfusion, which carries its own inherent risks including infection, transfusion reactions, and transfusion-associated immunosuppression (Demetri et al, 2001). Furthermore, the nation's blood supply is a limited resource (Sullivan et al, 2005). Thus, there is a well-defined need for safe and effective alternatives to transfusion in the setting of chemotherapy-induced anemia (CIA).

As the marketing authorization holder for both epoetin alfa and darbepoetin alfa, Amgen, in collaboration with Johnson & Johnson Pharmaceutical Research and Development LLC (J&JPRD), takes the responsibility for the safety of patients receiving these products very seriously. Amgen currently markets darbepoetin alfa (under the trade name Aranesp®) for use in the treatment of patients with CIA. Ortho Biotech Products, LP, under license from Amgen, currently markets epoetin alfa (under the trade name Procrit®) for the same indication. To date, over 1.6 million patient-years of postmarketing experience with darbepoetin alfa and over 4 million patient-years of postmarketing experience with epoetin alfa have been accumulated in the treatment of anemia in multiple therapeutic settings, including the approved indications of CIA and chronic renal failure.

ESAs have been shown to reduce the burden of transfusions and to effectively increase hemoglobin concentrations in patients with CIA in controlled clinical trials (Abels, 1992; Littlewood et al. 2001; Hedenus et al. 2002; Vansteenkiste et al. 2002). The efficacy and safety of ESAs have also been demonstrated for the treatment of anemia in other clinical indications, including chronic renal failure, human immunodeficiency virus (HIV) infection, and in patients scheduled to undergo elective, noncardiac, nonvascular surgery in order to reduce the need for allogeneic blood transfusions.

In all of the studies performed for purposes of supporting FDA labeling, the decision of when to transfuse patients was based on signs and symptoms of anemia and/or hemoglobin level, and was left to the discretion of the investigator. Thus, while the

regulatory hurdle established by FDA for approval and supplemental changes to labeling has been the objective, quantifiable endpoint of required red blood cell (RBC) transfusions, it must be borne in mind that such transfusions are prescribed to treat anemia, a clinical diagnosis based on signs and symptoms (Irvine et al, 1994; Vogelzang et al, 1997).

Over the past decade, the safety of ESAs in CIA has been demonstrated in thousands of patients participating in carefully monitored, placebo-controlled clinical trials. An increased risk of cardiovascular/thromboembolic (CV/TE) events has been consistently observed and appropriately represented in class labeling for all ESAs. However, based on concerns raised primarily by the publication of the ENHANCE and BEST studies (both conducted in non-CIA populations) and by more recent preliminary reports from the DAHANCA 10 study and Amgen Study 20010103, attention has focused on the question of whether ESA treatment may actually decrease survival in cancer patients, perhaps by promoting tumor progression. These potential risks were reflected in the prescribing information for all ESAs at the time of regulatory approval (with the theoretical risk of tumor progression added in the oncology labeling) and more recently were updated in the current labels for these products as a boxed warning. In addition, these risks were the focus of the 2004 ODAC, which supported monitoring of the safety of ESAs in ongoing investigator-sponsored clinical trials.

Risk assessment should be evidence-based and driven by a comprehensive analysis of all relevant data. To this end, Amgen and J&JPRD has provided for the 2007 ODAC meeting an updated risk assessment for darbepoetin alfa, epoetin alfa, and other ESAs in the oncology population.

Amgen has assembled for the ODAC review what we believe to be a complete analysis of all appropriate clinical studies in subjects with CIA. Rigorous combined analyses have been performed to evaluate the safety of darbepoetin alfa, epoetin alfa, and other ESAs in this population. Three types of analyses were performed: 1) a pooled analysis of data from individual study subjects including only data from Amgen-sponsored trials of darbepoetin alfa, 2) a meta-analysis of Amgen study-level darbepoetin alfa data, and 3) an updated meta-analysis based on the Cochrane Collaborative report (Bohlius et al, 2006) including relevant trials of ESAs conducted since the original Cochrane analysis. Individual results for key clinical studies are also presented. J&JPRD have also

assembled for the ODAC review a separate meta-analysis evaluating the safety of epoetin alfa in the treatment of CIA.

The high-level summary of the assembled weight of evidence is as follows:

(1) Preclinical data are reassuring with regard to the effect of ESAs on tumor progression and overall survival.

(2) Clinical data continue to indicate that ESAs are associated with an increased risk of venous thromboembolism. This risk has been accurately quantified and is reflected in the product labels.

(3) Comprehensive analyses of patient-level data from controlled clinical studies with darbepoetin alfa and epoetin alfa, when used in the setting of CIA, show a neutral effect on overall survival and tumor progression while demonstrating clear benefit in terms of reducing the need for blood transfusion.

(4) Updated meta-analyses of all ESAs involving over 8500 patients in 35 studies also demonstrate that the effect of ESAs on survival is neutral in patients with CIA (HR: 1.033, 95% CI: 0.922, 1.158).

(5) To date, four studies have been reported that show a significant, adverse effect on overall survival with ESA use in cancer: Epo-Can-20, BEST, ENHANCE and the 20010103 study of patients with active cancer not receiving chemo- or radiation therapy. The DAHANCA 10 study was stopped due to futility; definitive data from this study are awaited. All of these address experimental, unapproved indications.

(6) Only the 20010103 study and DAHANCA 10 are new since the 2004 ODAC meeting. In the same interval, four other new studies have shown neutral effects on survival: the 20040232 placebo controlled study in CIA across tumor types, the BRAVE controlled study in CIA in breast cancer, interim data from GELA study in CIA in NHL, and the Möbus controlled study in CIA in breast cancer.

(7) In CIA, the data presented at the 2004 ODAC concerning tumor progression and survival have become more extensive and robust. ESA administration does not appear to increase these risks in patients within this approved indication.

(8) Subject characteristic analyses cannot yet identify patients at special risk for adverse effects from ESA therapy. It is of interest that an achieved hemoglobin response predicts a favorable outcome, although this may represent simple confounding by patient status.

(9) The weight of evidence suggests that ESAs should not be used outside of the experimental setting to treat anemia associated only with active malignancy in patients who have exhausted other options, or as a strategy aimed at hyperoxic radiosensitization.

(10) The existing and substantial weight of evidence presented here supports the continued appropriate use of ESAs in CIA as per the prescribing information. Ongoing pharmacovigilance studies will further inform the risk/benefit assessment in the near future.

Amgen hopes to encourage a comprehensive scientific discussion of the available data and proposals for further initiatives to assist in risk assessment and minimization in this important area of cancer management.

This briefing document provides a detailed assessment of the identified risks of ESAs in the oncology setting, along with a description of the current risk management activities. The document is divided into the following key sections:

- An overview of marketed ESAs, including their clinical benefits, identified areas of risk, and risk management activities to date
- An updated risk assessment of ESAs in oncology reviewing all relevant, available safety data to date, including:
  - Darbepoetin alfa (Aranesp, Amgen): meta-analyses of all relevant safety data pertaining to survival, tumor progression, and CV/TE events in CIA
  - Darbepoetin alfa (Aranesp, Amgen): a review of data from Study 20010103 in anemic subjects with active cancer not receiving chemotherapy or radiotherapy (a non-indicated patient population)
  - Epoetin alfa (Procrit, Eprex, J&JPRD): meta-analyses of epoetin alfa safety data pertaining to tumor progression, survival, and CV/TE events in CIA, and a review of safety data on ESAs as an adjunct to radiotherapy (a non-indicated patient population)
  - All ESAs: an update of the Cochrane collaborative meta-analysis of all relevant safety data pertaining to survival in CIA

- An update regarding recently completed and ongoing risk management activities (including label updates and clinical studies) for both darbepoetin alfa and epoetin alfa
- An update of ongoing pharmacovigilance study activities.

As part of the current risk management plans for darbepoetin alfa and epoetin alfa, Amgen, the marketing authorization holder, and J&JPRD have addressed safety concerns through sequential updates to the product labeling, risk communications including Dear Health Care Professional letters, and ongoing pharmacovigilance programs. Data from these pharmacovigilance studies, as well as ongoing studies for other ESAs, should prove helpful in formulating future risk management activities.

2. Background

2.1 Erythropoietin and Physiological Response to Chemotherapy-induced Anemia

Erythropoietin (EPO) is a naturally-occurring glycoprotein hormone produced primarily in the adult kidney that regulates the production of RBCs to maintain the oxygen-carrying capacity of peripheral blood. Endogenous EPO is secreted, with mean (SD) plasma levels of 6.7 (1.7) mU/mL reported in healthy adults (Schena et al, 2002).

Anemia is a frequent complication of many diseases including chronic renal disease, or may be induced by iatrogenic factors such as myelosuppressive chemotherapy. In these conditions, anemia may result from defective production of, or an inadequate response to, endogenous EPO. Unlike anemic patients with renal failure where production of EPO may be impaired, anemic patients with cancer have increased levels of endogenous EPO relative to healthy subjects (Miller et al, 1990). For example, in an analysis of patients (n = 1122) with CIA (hemoglobin < 11 g/dL), the mean (SD) baseline endogenous EPO level was 81.9 (268) mU/mL with a range of 0.9 to 6440 mU/mL (Amgen data on file). These levels are, on average, approximately 10-fold higher as compared to endogenous EPO levels in normal subjects. Despite the relative increases in endogenous EPO, the levels achieved in this population are still lower than expected for the degree of anemia observed (Miller et al, 1990).

Recombinant human EPO (rHuEPO) and the longer-acting molecule, darbepoetin alfa, engage the same receptor through which endogenous EPO signals and trigger the same intracellular signaling pathways and the same physiological responses as endogenous EPO.

Mean serum rHuEPO concentrations of approximately 900 to 1300 mU/mL were reported in healthy adults and cancer patients following subcutaneous administration of 36000 or 40000 IU of rHuEPO (Cheung et al, 2001; Fujisaka et al 2006). These concentrations of rHuEPO are well within the range of endogenous EPO concentrations measured in cancer patients with anemia. Based on this analysis, treatment with ESAs would not be expected to raise EPO concentrations in patients to levels higher than those that are observed during the complex pathophysiological events of anemia related to cancer and its treatment.

2.2 Overview of Marketed ESAs

Amgen was the first company to clone the gene encoding epoetin alfa and is the marketing authorization holder (Sponsor) of the epoetin alfa Biologics License Application (BLA), approved in 1989 under the trade name EPOGEN®. Since April 1993, epoetin alfa has also been marketed by Ortho Biotech Products, LP under the name Procrit®, following FDA approval for its use in the treatment of anemia in patients with cancer receiving chemotherapy. Procrit/EPOGEN solution for injection (recombinant human erythropoietin, rHuEPO, epoetin alfa) is a glycoprotein manufactured by recombinant DNA technology, has an amino acid sequence identical to human urinary erythropoietin, is indistinguishable from naturally-occurring human erythropoietin on the basis of biological erythropoietic effects, and has a molecular weight of 30,400 daltons. Procrit and EPOGEN are identical except for the product label. EPREX is a closely related epoetin alfa product that is manufactured by an affiliate of J&JPRD in a separate facility under license from Amgen. NeoRecormon®/Recormon® (epoetin beta) is marketed by companies affiliated with Hoffmann-La Roche and has the same amino acid sequence as epoetin alfa.

Amgen also created and developed darbepoetin alfa (Aranesp®) and is the marketing authorization holder (Sponsor) of the initial BLA for the treatment of anemia associated with chronic renal failure including patients who are receiving dialysis (indication approved in 2001). Aranesp® (darbepoetin alfa) differs from epoetin alfa and epoetin beta at five amino acid positions, binds the EpoR less avidly, and has an appreciably longer serum-half life, permitting less-frequent dosing. Copies of the currently approved prescribing information for Procrit and Aranesp are included in Appendix 1.

2.3 Benefits of ESAs: Burden of Illness and Transfusions

The severity of anemia experienced by cancer patients depends in part on the extent of the underlying neoplastic disease, as well as the intensity of the cytotoxic treatments administered, with the degree of hematopoietic impairment being cumulative in nature. In patients with cancer, ESAs provide the only alternative to blood transfusion, which carries its own inherent risks. These risks are well recognized and have been acknowledged by FDA and other regulatory agencies.

2.3.1 Risks of Transfusions

2.3.1.1 Risk of Transfusion Reactions

Hemolytic transfusion reactions are rare but potentially serious. Approximately 1 in 1,000 patients has clinical manifestations of delayed reaction to transfusions; however, fatal acute hemolytic reactions occur in only 1 in 250,000 to 1 in 1 million transfusions, and are usually due to clerical error (AuBuchon, 2004; Linden et al, 1992; Linden et al, 2000).

Transfusion-related acute lung injury (TRALI) is similar to acute respiratory distress syndrome in presentation and is the leading cause of transfusion related morbidity and mortality worldwide. It occurs with an estimated frequency of 1 in 5,000 red cell transfusions, though the true incidence is probably higher (Silliman et al, 2005; Toy et al, 2005; Boshkov et al, 2005).

2.3.1.2 Risk of Infection

Although the risk of infection from diseases once frequently transmitted by blood, such as HIV, has been minimized, FDA acknowledges that blood supplies are constantly faced with new threats which typically require the development of new tests to keep blood safe (FDA News Press Release, 2006). A wide range of infectious diseases may be transmitted through allogeneic blood transfusion: risks for infection with HIV, hepatitis C, and hepatitis A are all in the range of 1 in 1 to 2 million (Dodd, 2003). The risk of infection with hepatitis B is significantly higher at 1 in 30,000 to 250,000 (Goodnough et al, 1999). Infections with human T-lymphotropic virus (HTLV) I and II, and parvovirus B19 are in a similar range (Schreiber et al, 1996; Dodd, 1994). The potential for infection with new and emerging pathogens like West Nile virus, severe acute respiratory syndrome, monkeypox, Trypanosoma cruzi, Plasmodium, Babesia, dengue virus, and variant Creutzfeldt-Jakob disease remains a concern (Alter et al, 2007; Pealer et al, 2003).

Although much attention has been given to iatrogenic viral transmission resulting from blood product transfusions, bacteremia resulting from contaminated blood is the second most frequently reported cause of death in transfusion patients after hemolytic reactions, and is responsible for more than 10% of all transfusion-associated deaths in the United States.

2.3.1.3 Risk of Thromboembolic events

Few studies have examined the effect of blood transfusions on the risks of developing thromboembolic (TE) events in patients undergoing surgery, and there is little concordance in the published literature. Observational studies have suggested an increase in thrombotic vascular events (TVEs) in patients receiving perioperative blood transfusions (Nilsson et al. 2007; Abu-Rustum et al, 2005; Gangireddy et al, 2007); however, this finding has not been consistently confirmed. The potential impact of RBC transfusions on thrombosis may be compounded in patients with cancer who are at an increased risk for thromboembolism compared with the general population as a result of their underlying disease (Alcalay et al. 2006; Blom et al. 2006; Chew et al. 2007).

2.3.2 Clinical Benefit of ESAs in CIA

Over the past decade, the clinical benefit of ESAs for improving hemoglobin and reducing the need for RBC transfusions in subjects with CIA has been repeatedly demonstrated (Glaspy et al. 1997; Demetri et al. 1998; Gabrilove et al. 2001; Littlewood et al. 2001; Hedenus et al. 2002; Vansteenkiste et al. 2002). A combined analysis of 57 trials and 9353 patients with cancer demonstrated that ESAs significantly reduced the risk of RBC transfusions (36% lower than control patients [RR = 0.64, 95% CI: 0.60 to 0.69; 42 trials and 6510 patients; $P < 0.001$]) and significantly improved hematologic responses compared with those observed in control patients (hemoglobin increase of 2 g/dL [RR = 3.43, 95% CI: 3.07 to 3.84; 22 trials and 4307 patients]) (Bohlius et al. 2006). The use of ESAs for the avoidance of RBC transfusions, as well as treatment of anemia in oncology patients, is now a part of standard clinical practice. Guidelines promulgated by independent clinical organizations such as the American Society of Hematology, American Society of Clinical Oncology, National Comprehensive Cancer Network, and European Organization for Research and Treatment of Cancer recommend the use of ESAs to maintain adequate hemoglobin levels (Rizzo et al, 2002; Bokemeyer et al, 2007; Rodgers, 2007).

Registration trials for the CIA indication were conducted using target hemoglobin levels ranging between 10 and 13 g/dL. In accordance with the current registered product labels, the dose of ESAs should be adjusted for each patient so that the hemoglobin level does not exceed 12 g/dL. Guidance is also provided regarding dose reductions based on hemoglobin levels and rate of rise. Recently, additional guidance has been

added to the label for ESAs to advise health care providers to maintain the lowest hemoglobin level sufficient to avoid the need for RBC transfusion (see Section 7.1).

Regulatory approval of ESAs by FDA for use in this and other anemic patient populations was achieved based on the completion and submission of randomized, blinded, controlled clinical studies using the objective and quantifiable endpoint of a reduction in the number of blood transfusions. This endpoint clearly was constructed to subsume the need to treat the signs and symptoms of anemia, which are well-documented quality of life (QOL) issues for these patients (Irvine et al, 1994; Vogelzang et al, 1997).

Systematic reviews of the published literature have consistently reported positive, albeit not definitive, effects of ESAs on health-related patient reported outcomes (Seidenfeld et al, 2006, Ross et al, 2006). These reviews include a report of the Agency for Healthcare Research and Quality (AHRQ), a federal agency that focuses on research on healthcare quality, costs, outcomes, and patient safety (Seidenfeld et al, 2006). Clinical guidelines continue to recommend ESAs for symptomatic patients with CIA (Rizzo et al, 2002; Bokemeyer et al, 2007), and clinical practice observations are highly consistent with improved QOL (Vogelzang et al, 1997).

2.4 Prior FDA and ODAC Assessments of Risk of ESAs in the Oncology Indication

The theoretical risk of tumor progression with ESA treatment in the oncology patient population has been acknowledged in the prescribing information since 1993.

In response to this putative risk, J&JPRD evaluated the potential for stimulatory effects on epoetin alfa on solid tumor growth as a post-approval commitment (Study N93-004). Study N93-004 was a double-blind, placebo-controlled study designed to enroll subjects with newly-diagnosed limited or extensive stage small cell lung cancer who were to be treated with etoposide and cisplatin, and investigated the potential for progression in solid tumors. Although median survival time and overall survival were similar in the 2 treatment groups, and tumor response and survival through month 12 appeared similar, beyond month 12, there was divergence in the survival curves favoring the placebo group. Data, however, are sparse for this period and complete follow-up information is not available. This commitment was discharged in agreement with FDA in May 2004 following study termination due to poor patient accrual (see Section 7.3.1).

Two studies conducted outside the US suggesting that treatment with Eprex (epoetin alfa; INT-76 or BEST) and NeoRecormon (epoetin beta; ENHANCE) had a negative effect on progression-free survival and survival in patients with metastatic breast cancer and a negative effect on locoregional control and survival in patients with advanced squamous cell carcinoma of the head and neck, respectively, were reported in 2003 (Henke et al, 2003, Leyland-Jones et al, 2003). In both of these studies, patients were treated to achieve hemoglobin levels greater than 12 g/dL; target hemoglobin concentrations were > 12.0 g/dL and < 14.0 g/dL for BEST (Leyland-Jones et al, 2003) and ≥ 14.0 for women and ≥ 15.0 g/dL for men for ENHANCE (Henke et al, 2003).

Also in 2003, J&JPRD informed FDA of the termination of 3 clinical studies due to an increase in the frequency of deep venous thrombosis relative to a placebo control (PR00-03-06 [gastric or renal cancer]) or relative to previously controlled studies (PR01-04-005/GOG-0191 [cervical cancer] and EPO-CAN-15 [limited disease small cell lung cancer]). J&JPRD also informed FDA of 2 additional studies that were terminated by the Data Monitoring Committee (DMC) after unplanned interim analyses due to a non-significant trend toward lower locoregional control and an imbalance in survival favoring the control group in subjects with head and neck cancer (RTOG-9903) and an apparent imbalance in survival favoring the control group in subjects with nonsmall cell lung cancer (EPO-CAN-20). Further details on RTOG-9903 are provided in Section 5.2.2.1.

In May 2004 an ODAC meeting was convened to address the safety of ESAs in patients with cancer. The BEST and ENHANCE studies and the potential safety risks associated with ESA treatment of patients with cancer above hemoglobin levels recommended in the prescribing information were the focus of the data reviewed. These findings were unexpected based on the previous accumulated preclinical and clinical experience with ESAs. The concerns raised by these studies prompted comprehensive analyses of all relevant data available for epoetin alfa, epoetin beta, and darbepoetin alfa. Several pooled analyses retrospectively examining safety and survival data from large, placebo-controlled trials were presented at the meeting and are reported in the Company Briefing Documents available on the FDA Advisory web site (http://www.fda.gov/ohrms/dockets/ac/04/briefing/4037b2.htm).

Conclusions of the 2004 ODAC Committee and Subsequent Risk Management Activities

Given the lack of definitive clinical data suggesting adverse effects on overall survival or tumor progression associated with ESA use in patients with CIA (the labeled indication), it has been suggested that the outcomes of the BEST and ENHANCE studies may have been related to the unique design features of those trials. In particular, the high hemoglobin entry criteria and target hemoglobin > 12 g/dL could perhaps have contributed to an increased risk of CV/TE events.

FDA commented at the 2004 ODAC that the dosing recommendations at that time were adequate to minimize the risks of thrombotic events. However, in agreement with FDA, Amgen (Aranesp) and J&JPRD (Procrit) later updated the label information in December 2004 to inform prescribers of the cardiovascular risks and potential for tumor progression. Specifically, results from the BEST trial were included in the WARNINGS section, as follows:

> *Higher risk treatment with epoetin alfa was associated with a higher rate of fatal thrombotic events (1.1% epoetin alfa versus 0.2% placebo) in the first 4 months of the study. Mortality at one year, the primary endpoint of the study, was higher for the epoetin alfa group (76% epoetin alfa versus 70% placebo, p = 0.012)*

In the PRECAUTIONS section, the risk of tumor progression was described with regard to both the BEST and ENHANCE study results. In reference to the BEST study:

> *Mortality at 12 months was significantly higher in the epoetin alfa arm. This difference was observed primarily in the first 4 months of the study with more deaths attributed to breast cancer progression in the epoetin alfa group (6% epoetin alfa versus 3% placebo)*

A reference to the ENHANCE study was also included:

> *Locoregional progression-free survival was significantly shorter (median of 406 days Epoetin beta vs 745 days placebo, p = 0.04) in patients receiving Epoetin beta*

The label also reflected the fact that there was insufficient information to establish whether use of ESAs have an adverse effect on time to tumor progression or progression-free survival.

Upon agreement with FDA on the approved label changes, a corresponding Dear Health Care Professional letter was sent by both companies to communicate the new safety information added to the prescribing information.

With respect to clinical trial activities, ODAC supported further investigation of the risk of tumor progression in the form of randomized placebo-controlled studies to evaluate the potential for tumor growth, including the use of data derived from clinical trials presented by Amgen and J&JPRD at the meeting. FDA noted that such data for US-approved ESAs should ideally be at licensed doses.

Since that time, Amgen has also continued to monitor five randomized, prospective clinical trials of darbepoetin alfa as part of the Aranesp Pharmacovigilance Program (see Section 7.2). These studies were presented at the 04 May 2004 ODAC meeting and, following agreement with the FDA, the requirement to report the data from these studies was identified in 2006 as formal post-marketing commitment studies. None of these studies were originally designed as part of a formal pharmacovigilance program, but were proposed by Amgen as a means of taking advantage of ongoing studies to explore any possible effects of darbepoetin alfa on overall survival in patients with CIA. This approach had the advantage of permitting the acquisition of informative data in a relatively short timeframe. An update on the status of these studies is provided in Section 7.2 of this document.

Similarly, as part of its ongoing risk management activities, J&JPRD agreed to provide the FDA with periodic safety updates from five ongoing, randomized Eprex studies that were also presented to the 2004 ODAC and which included survival as an endpoint (EPO-GBR-7, EPO-GER-22, AGO/NOGGO, EPO-CAN-17, and the Möbus Study) (see Section 7.3). As noted previously, following the termination of the postmarketing commitment Study N93-004, J&JPRD was requested by FDA to conduct a new study. This phase 4 study (EPO-ANE-3010) is currently ongoing in subjects with metastatic breast cancer with the objective of addressing the question of whether ESAs adversely affect breast cancer outcomes in patients receiving treatment according to the product label. Updates on these studies are provided in Section 7.3 of this document.

2.5 Safety Concerns Raised by Recent Clinical Studies

Survival and Tumor Progression

Two recent studies have caused FDA to reexamine the previously identified safety concerns of tumor progression, survival, and CV/TE events in cancer patients in investigational settings. These studies were conducted in patients with head and neck cancer receiving radiation therapy (DAHANCA 10) and anemic subjects with active cancer who were not receiving chemotherapy or radiotherapy (Amgen Study 20010103).

In December 2006, the DAHANCA Study Group notified Amgen and study investigators of preliminary interim results of the DAHANCA 10 study (described in Section 7.2.5), 1 of 4 investigator-sponsored studies contributing to the Aranesp pharmacovigilance program. The preliminary interim data for this open-label study were reported to have indicated an approximate 10% difference in 3-year locoregional control ($p = 0.01$) in favor of the control group. Overall survival showed a smaller, nonsignificant difference in favor of the control group ($p = 0.08$). Darbepoetin alfa treatment was apparently not associated with any excess serious adverse events in this preliminary interim analysis. Based on these preliminary interim results, the DAHANCA group concluded that the trial would be unlikely to demonstrate improved outcomes with darbepoetin alfa treatment, and enrollment into the study was terminated. Long term follow-up of the 522 patients already enrolled is continuing. Data beyond these preliminary high-level reports are currently unavailable pending data review, analysis, and publication by the principal investigator. Amgen is providing support to facilitate this process.

In January 2007, final results became available from a phase 3, randomized, double-blind, placebo-controlled trial of darbepoetin alfa in anemic subjects with active cancer who were not receiving chemotherapy or radiotherapy (Amgen Study 20010103), an indication distinct from CIA and not approved for use in the prescribing information. Although this study was not designed to determine the effect of darbepoetin alfa on survival or to assess the cause of death, more deaths occurred on study in the darbepoetin alfa treatment group (26.4%) compared with the placebo group (20.0%). In an analysis of overall survival (including long-term follow up), the hazard ratio of time to all deaths in the darbepoetin alfa group relative to the placebo group was 1.29 (95% CI: 1.08, 1.55) ($p = 0.006$), based on the Cox regression analysis stratified by the factors

used at randomization but unadjusted for any additional prespecified covariates. Additional information on these results is provided in Section 4.3.

As a result of the above-mentioned studies, FDA requested that Amgen and J&JPRD amend the current prescribing information and provide a boxed warning in the labeling for darbepoetin alfa and epoetin alfa (see Section 7.1). The safety concerns raised by these studies are also the subject of the current ODAC meeting.

Death and Serious Cardiovascular Events

In April 2006, J&JPRD made FDA aware of results from a randomized, prospective trial entitled “Correction of Hemoglobin and Outcomes in Renal Insufficiency” (CHOIR). The CHOIR study evaluated 1432 anemic chronic renal failure patients who were not undergoing dialysis. Patients were assigned to epoetin alfa treatment targeting a maintenance hemoglobin concentration of 13.5 g/dL or 11.3 g/dL. A major cardiovascular event (death, myocardial infarction, stroke or hospitalization for congestive heart failure) occurred among 125 (18%) of the 715 patients in the higher hemoglobin group compared to 97 (14%) among the 717 patients in the lower hemoglobin group (HR 1.3, 95% CI: 1.0, 1.7, p = 0.03).

In light of the above-mentioned study, labeling was changed and FDA issued a public health advisory in November 2006.

3. Preclinical Evidence of Risk With ESAs

The theoretical possibility that ESAs could act as growth factors was acknowledged at the time of FDA approval of epoetin alfa for the treatment of anemia in the oncology setting, and is included in the currently approved US product label for all ESAs. Data on this topic were reviewed at the 2004 ODAC meeting. A systematic review of the literature was conducted to characterize the available evidence in 2004, and an updated review has been assembled.

The hypothesis that ESAs promote tumor growth through interaction with the EPO receptor (EpoR) has been extensively investigated. Amgen has carefully reviewed the available literature to objectively examine the level of evidence that support this hypothesis. The key points of a review provided in Appendix 2 are summarized below

It has been hypothesized that rHuEpo may directly promote tumor growth via an interaction with EpoR expressed on the surface of tumor cells. Putative EpoR expression has been characterized by several methods in a variety of human tumors and tumor cell lines. These findings must be considered in light of problems with EpoR-detection methodologies, conflicting data from different groups, the lack of direct correlation between reported expression of EpoR protein and the presence of the receptor on the surface of tumor cells, and the lack of tumor-promoting activity by ESAs in animal tumor models. We note the following points:

- EpoR is not an oncogene. The EpoR gene is not significantly amplified or overexpressed in solid tumors and overexpression of constitutively activated mutant forms of EpoR does not transform cells (Sinclair et al., 2005; Longmore & Lodish, 1991). One would expect such behavior if Epo-induced signaling could drive proliferation of cancer cells.
- EpoR hyperactivating mutations result in polycythemia and are not a feature of malignancy. Similarly, in clinical conditions in which Epo is overexpressed (eg, Chuvash polycythemia) or in which EpoR signaling is not controlled (EpoR truncations), polycythemia results, but with no increase in tumor incidence (Arcasoy et al., 2002; Gordeuk et al., 2004; de la Chapelle et al., 1993). While this could result from the relatively narrow expression pattern of the EpoR, the fact that EPO overexpression in humans is not associated with malignancy is reassuring.

- The EpoR gene is transcribed in most tissues and cell lines at low to moderate levels. Levels of EpoR mRNA are rarely elevated in tumors and cell lines above that seen in the normal tissue of tumor origin (Sinclair et al., 2005; Feldman et al., 2006; Winter et al., 2005). Again, molecules that deliver proliferative signals are frequently expressed at high levels by at least some tumors. This is not the case with the EpoR.
- Attempts to demonstrate EpoR protein expression are confounded by the fact that all commercially available anti-EpoR antibodies are non-specific and are unsuitable for immunohistochemistry. We and others have demonstrated that the most commonly used EpoR polyclonal antibody (Santa Cruz C-20) detects heat shock protein HSP70, not EpoR, in tumor samples (Brown et al, 2007; Elliot et al, 2006; Osterborg et al., 2007).
- EpoR mRNA levels do not necessarily correlate with fully functional EpoR protein levels, reflecting both usual variations in translational fidelity as well as the inability of most routinely-employed methods to distinguish between alternatively spliced forms of the EpoR mRNA that encode proteins with attenuated or antagonistic functions (Nakamura et al., 1992; Arcosoy et al., 2003).
- As is expected when analyzing receptor molecules, EpoR protein synthesis does not necessarily correlate with cell surface expression or signaling of the EpoR. Less than 1% of EpoR normally gets to the surface of the cell due to inefficient processing, protein degradation, requirements for limiting accessory molecules for trafficking to the surface (e.g. Jak2), requirements for limiting accessory molecules for intracellular signaling, and because of its short cell-surface half life (Suspino-Rosin et al., 1999; Huang el al., 2001; Hermine et al., 1996).
- Studies that investigate the direct role of Epo:EpoR in signaling, proliferation, migration, and survival of cancer cells have not yielded conclusive results. For example, the majority of in vitro studies that report an effect have used levels of rHuEpo (>10 U/mL) that are unattainable in patients. Even so, the effects of such treatments are modest (Gewirtz et al, 2006; Hardee et al, 2006; Pajonk et al, 2004).
- All rodent tumor models (23 independent studies) have demonstrated that ESAs do not enhance tumor growth. To the contrary, ESAs have been shown to

increase sensitivity of tumor cells to radiation or chemotherapy. (Sigounas et al; 2004; LaMontagne et al, 2006; Shannon et al, 2005).

- ESAs do not mediate any consistent adverse effect on tumor angiogenesis in rodent tumor models (Ceelen et al, 2007; Hardee et al, 2005; Tovari et al, 2005; Pinel et al, 2004).
- The data do not support a meaningful effect of ESAs on mobilization of endothelial progenitor cells (Heeschen et al, 2003 ; Hirata et al, 2006 ; and Prunier et al, 2007), nor are there compelling data that such cells, if mobilized, play a meaningful role in tumor vascularization either in preclinical models or in patients (Asahara et al, 1999; Natori et al, 2002; Machein et al, 2003; Gothert et al, 2004).

It should be noted that a substantial body of preclinical data associates ESA treatment with improved outcomes in experimental anti-cancer therapies. It is well established that lower doses of ionizing radiation are required for tumor ablation if the oxygen tension within tumors is high. Higher hemoglobin levels induced by ESA treatment are associated with improved responses to radiotherapy or chemotherapy in preclinical tumor models (Kelleher et al, 1996; Pinel et al, 2004). Moreover, increased tumor oxygenation reduces hypoxia-regulated VEGF levels and consequently tumor angiogenesis (Dunst et al, 2002; Leyland-Jones et al, 2003).

Taken together, these results provide substantial reassurance that ESAs should not meaningfully stimulate tumor cell proliferation either directly, or through a secondary effect on host tissues. A recently published, independent review of the relevant literature (Osterborg et al., 2007) reached essentially the same conclusion.

4. Risk Assessment of Darbepoetin alfa, Epoetin alfa, and Other ESAs in Oncology Based on Amgen Analyses

In addition to the review of the preclinical literature, Amgen has conducted a clinical risk assessment of ESAs in the oncology setting, including evaluation of the following areas:

- clinical evidence regarding the risk of darbepoetin alfa, epoetin alfa, and other ESAs in CIA
- clinical evidence regarding the risk of darbepoetin alfa in cancer-related anemia in the absence of chemotherapy or radiotherapy– an unapproved patient indication (Study 20010103)

4.1 Analytic Methodology

4.1.1 Overview of Studies Included in the Analysis

To evaluate the risk/benefit of darbepoetin alfa, a review of all Amgen-sponsored, well-controlled, randomized clinical trials in oncology patients was conducted. Studies considered for inclusion in the analysis were completed trials in which darbepoetin alfa was administered SC in subjects with CIA. Studies included in the combined analysis are listed in Appendix 3.

Amgen-sponsored studies in oncology meeting the above criteria that were not included in the combined analysis were anemia of cancer studies, single-arm trials in CIA, and studies in CIA in which approximately the same dose was evaluated in all treatment groups. These studies are listed in Appendix 4, and safety data for these studies are provided in Appendix 5.

The analyses were limited to studies that included subjects with CIA with or without radiotherapy, as listed in Section 4.2. CIA was defined as a hemoglobin ≤ 11 g/dL at study screening in subjects with nonmyeloid tumors who had received at least 1 cycle of chemotherapy and were scheduled to receive future chemotherapy. Studies were analyzed as follows:

- randomized, blinded, placebo-controlled studies
- randomized studies with a standard-dose ESA active control group (excluding placebo-controlled studies)

4.1.2 Adverse Events of Interest

Three important areas of potential increased risk were analyzed: survival, progression-free survival (tumor progression), and CV/TE events.

Analyses of adverse event data reported in Amgen clinical trials were recoded using a standard dictionary, MedDRA version 9. A prespecified list of preferred terms was used to identify adverse events of interest (eg, CV/TE; seizures; hypertension; pure red cell aplasia; immune system disorder; neoplasms benign and malignant) and a SAS macro was developed to identify these adverse events based on the preferred terms assigned to reported events. Adverse events were selected without regard to severity of event (all severities are included) and reported relationship to investigational product.

Deaths on study were identified on case report forms as either the reason for study drug or study termination or as fatal adverse events (ie, grade 5 events). If a death was identified using more than one method, the time to death was based on the earliest date reported. Collection of disease progression information has not been an objective of darbepoetin alfa oncology studies to date; specific criteria for its evaluation (eg, timing, methods) were not included in any of the study protocols. Therefore, disease progression was identified: 1) if the reason for ending study drug or the study was reported as "disease progression," or 2) if the end of study disease status was determined to be "progressive disease" based on the investigator's evaluation. If disease progression was identified using more than one method, then the time to disease progression was based on the earliest date reported (ie, date of study drug termination, study termination, or end of study disease status evaluation). Progression-free survival was calculated based on information on death or disease progression, and the time was based on date of progression or date of death, whichever was earlier.

4.1.3 Overview of Approach to Meta-analysis

Three different meta-analysis approaches were used for combining data across studies:

- A pooled analysis of patient-level data from Amgen clinical trials (darbepoetin alfa studies)
- A study-level meta-analysis of Amgen clinical trials (darbepoetin alfa studies)
- A meta-analysis of study level data (for all ESAs) supplementing data in the Cochrane Collaborative analysis

Pooled Patient-level Data Analysis of Amgen Clinical Trials

Data for individual subjects within identified studies were combined into pooled analysis data sets, that included patient identification number, study protocol, treatment group, and demographic/disease characteristic information. Since the duration of studies

varied, efficacy endpoints were evaluated at week 13 as well as at the end of treatment (as defined in individual protocols) and safety analyses included exposure adjusted estimates of adverse events as well as hazard ratio estimates from a Cox regression analysis (which includes study as a stratification factor). Results of the exposure adjusted analyses of adverse events were similar to the incidence of adverse events; only the analysis of incidence of adverse events is included in this document. Common definitions used for this analysis were prespecified prior to beginning the analysis.

Both efficacy and safety data were analyzed for all subjects who received at least one dose of investigational product by randomized treatment group. As a secondary analysis for safety, data were analyzed for all subjects who received at least one dose of investigational product by treatment received, as defined within each individual study (safety treatment group). Since results between the two analyses were consistent, only analyses by randomized treatment group are included in this document.

The pooled patient-level data analysis of placebo-controlled studies also included an examination of risk factors that could potentially influence the occurrence of the endpoints analyzed. This was done by calculating the unadjusted hazards ratio (HR) using a Cox-proportional hazards regression that included only treatment group in the model, then comparing this to the HR adjusted for a variety of risk factors thought to impact survival and safety endpoints; both types of models were stratified by study protocol to adjust for study-specific differences, such as allocation ratio. The majority of these potential risk factors were covariates that were ascertained at study baseline: age group, sex, weight category, hemoglobin category, ECOG score category, FACT-Fatigue (FACT-F) score category, endogenous serum erythropoietin (EPO) category, disease stage, and history of thrombosis, hypertension, or other cardiovascular diseases. Baseline glomerular filtration rate (based on serum creatinine level) and time since cancer diagnosis were also considered; however, these covariates were missing in >12% of subjects and were subsequently excluded from the analysis. In addition, the effects of 4 post-dosing factors were also explored for subjects receiving darbepoetin alfa only: hemoglobin > 12 g/dL, hemoglobin > 13 g/dL, a rise in hemoglobin that exceeded 1 g/dL in 14 days, and receipt of at least 1 RBC transfusion. These factors were included in additional Cox proportional hazards models as time dependent covariates (based on the time to first occurrence) and are presented separately.

Study-level Meta-analysis of Amgen Clinical Trials

A study-level meta-analysis limited to Amgen clinical trials was done using Comprehensive Meta Analysis software (Version 2.2.040). Adverse events of interest (cardiovascular and thrombotic events; embolism/thrombosis events) and on-study deaths were summarized for all Amgen clinical trials. Heterogeneity between studies was evaluated using a chi-square test (Q) to assess the probability that the differences in treatment effects between studies is due to chance (significance level set at 0.10) and quantifying inconsistency with the I^2 statistic (where $I^2 = 100\% \times [Q - df]/Q]$). Studies were grouped in a similar manner to the groups described for the pooled patient-level data analysis.

Updated Cochrane Analysis of Study-level Data

The Cochrane Collaborative report (specifically, Analysis 05.05) was used as the basis for an updated meta-analysis of published trial results for death. Two modifications were made to the Cochrane Collaborative analytic approach. In the Cochrane Collaborative analysis, studies were grouped by cancer therapy modality; the current analysis is limited to studies where chemotherapy was given. An additional modification was that the Cochrane Collaborative analyzed studies that allowed at least some platinum chemotherapy separately from those that excluded all use of platinum chemotherapy; our reanalysis collapsed these 2 groups into a single group referred to as the CIA group. Published data on deaths from relevant studies that have become available since the Cochrane Collaborative report was generated were added to this analysis. For these analyses, calculation of the odds ratio was based on the Peto method, which is the same analytic method described in the original Cochrane Collaborative report. Since Peto's method may results in biased estimates when there is a severe imbalance in sample size between the treatment groups. The Mantel-Haenszel method of computing the risk ratio was also calculated as a sensitivity analysis.

In addition to using Peto's fixed effects model, random effects meta-analyses were also conducted using the method described by DerSimonian and Laird. The results using a random effects model were similar to those using the fixed effects model; therefore, only the results of the fixed effects model are presented in this document.

Results from the following studies were added or updated for the analysis of CIA: Aapro et al, 2006; Blohmer et al, 2004; Grote et al, 2005; J&JPRD study EPO-GER-22; Razzouk et al, 2006; Savonije et al, 2005; Taylor et al, 2005; and the Möbus study. These studies were selected using the same criteria as those used by the Cochrane Collaborative group, and included:

> "… all randomized, controlled trials comparing epoetin or darbepoetin plus red blood cell transfusion with red blood cell transfusion alone for prophylaxis or treatment of anemia in cancer patients with or without concurrent antineoplastic therapy to prevent or reduce anemia. Control groups of included studies received identical antineoplastic and supportive treatments. Ongoing and small studies (≤ 10 subjects per study arm) were excluded."

The results of the interim analysis of the DAHANCA 10 study are not included in this analysis, as the data currently available are incomplete and could not be combined with data from other studies. This study is discussed in Section 7.2.2. The GELA study also was not included because patients in the control arm who had symptomatic anemia had the opportunity to receive another ESA according to local practice. This study is also discussed in Section 7.2.2.

Wherever possible, the original source documents were reviewed to confirm values used in the meta-analysis. Differences, if found, were summarized (data on file at Amgen). Results are presented as forest plots of all studies using the estimated ratio and the lower and upper 95% confidence limits. This analysis was also done using Comprehensive Meta Analysis software.

4.2 Assessment of Darbepoetin alfa in the Treatment of Chemotherapy-induced Anemia

The primary patient-level and study-level analyses presented include data from 6 Amgen-sponsored, randomized, double-blind, placebo-controlled trials in CIA conducted in 1515 subjects receiving darbepoetin alfa (n = 901) or placebo (n = 614). Information on these studies is provided in Table 1 and Table 2.

Table 1. Amgen-sponsored, Randomized, Double-blind, Placebo-controlled Studies Evaluating Darbepoetin alfa in Subjects With Chemotherapy-induced Anemia

Study	Phase	Study title	Darbepoetin alfa Starting Dose and Schedule	Duration of Treatment
980291 Schedule 1 (n = 249)[a]	2	A randomized, double-blind, placebo-controlled, dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous (SC) injection for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy	4.5, 6.75, 9.0, 12.0, 13.5, 15.0 μg/kg Q3W	12 weeks
980291 Schedule 2 (n = 156)[a]	2	A randomized, double-blind, placebo-controlled, dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous (SC) injection for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy	9.0, 12.0, 15.0, 18.0 μg/kg Q4W	12 weeks
990114 (n = 66)[a]	2	A multi-centre, blinded, placebo-controlled, randomised, dose finding study of NESP administered by SC injection for the treatment of anaemia in subjects with lymphoproliferative malignancies receiving chemotherapy	1.0, 2.25, 4.5 μg/kg QW	12 weeks
980297 (n = 314)[a]	3	A double-blind, placebo-controlled, randomised study of novel erythropoiesis stimulating protein (NESP) for the treatment of anaemia in lung cancer subjects receiving multicycle platinum-containing chemotherapy	2.25 μg/kg QW	12 weeks
20000161 (n = 344)[a]	3	A multicenter, blinded, placebo-controlled, randomized study of novel erythropoiesis stimulating protein (NESP) for the treatment of anemia in subjects with lymphoproliferative malignancies receiving chemotherapy	2.25 μg/kg QW	12 weeks
20030232 (n = 386)[a]	3	A randomized, double-blind, placebo-controlled study of darbepoetin alfa for the treatment of anemia in subjects with non-myeloid malignancy receiving multicycle chemotherapy	300 μg Q3W	15 weeks

[a] Number of subjects randomized who received at least 1 dose of investigational product

Table 2. Subjects by Study and Treatment Group (Placebo-controlled CIA Studies, Randomized Group)

	Total (N = 1515)
All placebo-controlled CIA Studies - n(%)	1515 (100)
Placebo	614 (40.5)
DA	901 (59.5)
980297	314 (20.7)
Placebo qw	158 (10.4)
DA 2.25 mcg/kg qw	156 (10.3)
20000161	344 (22.7)
Placebo qw	170 (11.2)
DA 2.25 mcg/kg qw	174 (11.5)
20030232	386 (25.5)
Placebo q3w	193 (12.7)
DA 300 mcg/kg q3w	193 (12.7)
980291 S1	249 (16.4)
Placebo q3w	51 (3.4)
DA q3w	198 (13.1)
4.5 mcg/kg	32 (2.1)
6.75 mcg/kg	17 (1.1)
9 mcg/kg	46 (3.0)
12 mcg/kg	28 (1.8)
13.5 mcg/kg	35 (2.3)
15 mcg/kg	40 (2.6)
980291 S2	156 (10.3)
Placebo q4w	31 (2.0)
DA q4w	125 (8.3)
9 mcg/kg	31 (2.0)
12 mcg/kg	31 (2.0)
15 mcg/kg	33 (2.2)
18 mcg/kg	30 (2.0)
990114	66 (4.4)
Placebo qw	11 (0.7)
DA qw	55 (3.6)
1 mcg/kg	11 (0.7)
2.25 mcg/kg	22 (1.5)
4.5 mcg/kg	22 (1.5)

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4.2.1 Demographics and Baseline Characteristics

Demographic characteristics were similar between groups in the patient-level analysis. Slightly more women than men were included in the analysis in both the darbepoetin alfa group (54.6% versus 45.4%) and the placebo group (52.0% versus 48.0%). Most subjects were white (92.1% darbepoetin alfa, 92.5% placebo). The mean (SD) age was similar in the darbepoetin alfa group (62.3 [12.3] years) and the placebo group (62.3 [11.8] years). Approximately half of the subjects in each group were ≥ 65 years of age (48.3% darbepoetin alfa, 46.9% placebo).

The most frequent primary tumor types in both treatment groups were lung and hematologic cancers, reflecting the two large, phase 3 studies (980297 and 20000161) conducted in these patient populations (Table 3). These tumor types accounted for 54.2% of subjects in the darbepoetin alfa group and 69.1% of subjects in the placebo group. Most subjects had later stage disease, defined as stage III or higher/extensive (81.9% darbepoetin alfa, 78.8% placebo). Overall, 77.4% of subjects in the darbepoetin alfa group and 71.7% of subjects in the placebo group had an ECOG status of 0 or 1. A high percentage of subjects in both groups did not have an investigator assessment of disease status at baseline, as assessment of disease progression was not an objective for any of the studies included in the analysis.

Table 3. Disease Characteristics
(Placebo-controlled CIA studies, Randomized Group)

	Placebo (N=614)	NESP (N=901)	TOTAL (N=1515)
Current Disease Stage - n(%)			
Stage II or Lower/Limited	99 (16.1)	128 (14.2)	227 (15.0)
Stage III or Higher/Extensive	484 (78.8)	738 (81.9)	1222 (80.7)
Other	17 (2.8)	23 (2.6)	40 (2.6)
Unknown	14 (2.3)	12 (1.3)	26 (1.7)
ECOG performance status - n(%)			
0	129 (21.0)	228 (25.3)	357 (23.6)
1	311 (50.7)	469 (52.1)	780 (51.5)
2	87 (14.2)	98 (10.9)	185 (12.2)
3	6 (1.0)	9 (1.0)	15 (1.0)
Unknown	81 (13.2)	97 (10.8)	178 (11.7)
Disease Status			
Complete response	5 (0.8)	5 (0.6)	10 (0.7)
Partial response	46 (7.5)	48 (5.3)	94 (6.2)
Stable disease	112 (18.2)	108 (12.0)	220 (14.5)
Progression	133 (21.7)	135 (15.0)	268 (17.7)
Not evaluable	63 (10.3)	63 (7.0)	126 (8.3)
Not evaluated	255 (41.5)	542 (60.2)	797 (52.6)
Tumor Category - n(%)			
Breast	62 (10.1)	144 (16.0)	206 (13.6)
Gastrointestinal	56 (9.1)	106 (11.8)	162 (10.7)
Genitourinary	10 (1.6)	41 (4.6)	51 (3.4)
Gynecologic	31 (5.0)	81 (9.0)	112 (7.4)
Hematologic	217 (35.2)	255 (28.3)	472 (31.2)
Lung	207 (33.6)	233 (25.9)	440 (29.0)
Other	31 (5.0)	41 (4.6)	72 (4.8)

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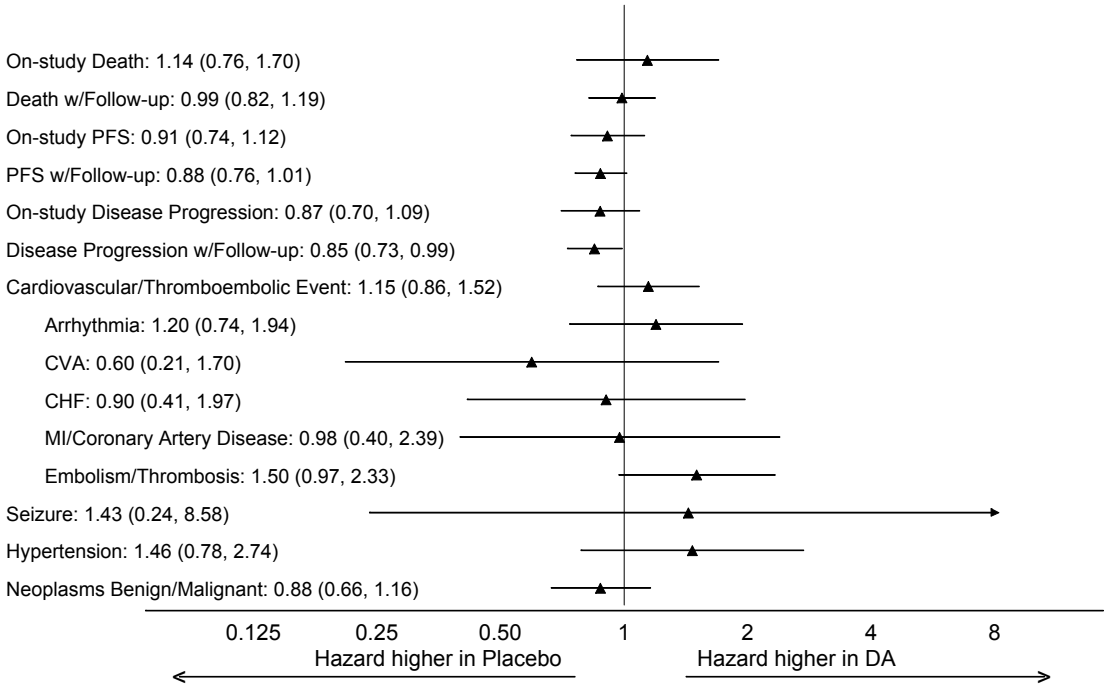
4.2.2 Overall Summary of Risk for Darbepoetin alfa in Chemotherapy-induced Anemia

A forest plot of hazards ratios (HR) for deaths, disease progression, and adverse events of interest based on the patient-level pooled analysis for placebo-controlled studies in subjects with CIA (901 darbepoetin alfa, 614 placebo) is shown in Figure 1. The point

estimate for death was higher in the darbepoetin alfa group than in the placebo group when on-study deaths were analyzed, with a CI that spanned 1 (HR: 1.14; 95% CI: 0.76, 1.70) (Figure 1). However, when deaths during the follow-up period were included, the point estimate comparing darbepoetin alfa to placebo was essentially 1 (HR: 0.99; 95% CI: 0.82, 1.19) (Figure 1). Disease progression and the composite endpoint of disease progression or death (referred to as progression-free survival or "PFS" in this analysis) revealed neutral outcomes regardless of the time period analyzed. Consistent with these data, the general adverse event category of Neoplasms Benign or Malignant was also reported at a lower rate in the darbepoetin alfa group compared to the placebo group (HR: 0.88; 95% CI: 0.66, 1.16) (Figure 1).

Figure 1. Adverse Event Hazard Ratios
(Placebo-controlled CIA Studies, Randomized Group)



Immune System Disorder and Pure Red Cell Aplasia have been removed from this figure.

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Overall reported CV/TE events occurred more frequently in the darbepoetin alfa group compared to the placebo group (HR 1.15; 95% CI: 0.86, 1.52); this result was likely heavily influenced by the subcategory of TE events, which had a HR of 1.50 (95% CI: 0.97, 2.33). The incidence of TE events, primarily representing venous

thromboembolism, was 7.8% in the darbepoetin alfa group and 5% in the placebo group; this incidence is consistent with the rates previously reported. These events were primarily venous rather than arterial. Of note, cardiovascular events such as myocardial infarction, cerebrovascular accident, and congestive heart failure were not observed at a higher rate in subjects receiving darbepoetin alfa for CIA.

Hypertension was also reported at a higher frequency in the darbepoetin alfa group compared to the placebo group (HR 1.46; 95% CI: 0.78, 2.74) (Figure 1). Again, the incidence was similar to those previously reported, with 3.4% of the darbepoetin alfa and 2.6% of the placebo group reporting hypertension of any grade.

Although the HR for seizure was also 1.43, the confidence interval was wide (95% CI: 0.24, 8.58) (Figure 1), as this event was reported in very few subjects (0.3% in both treatment groups).

Further analyses for each of these event categories are provided in the following sections.

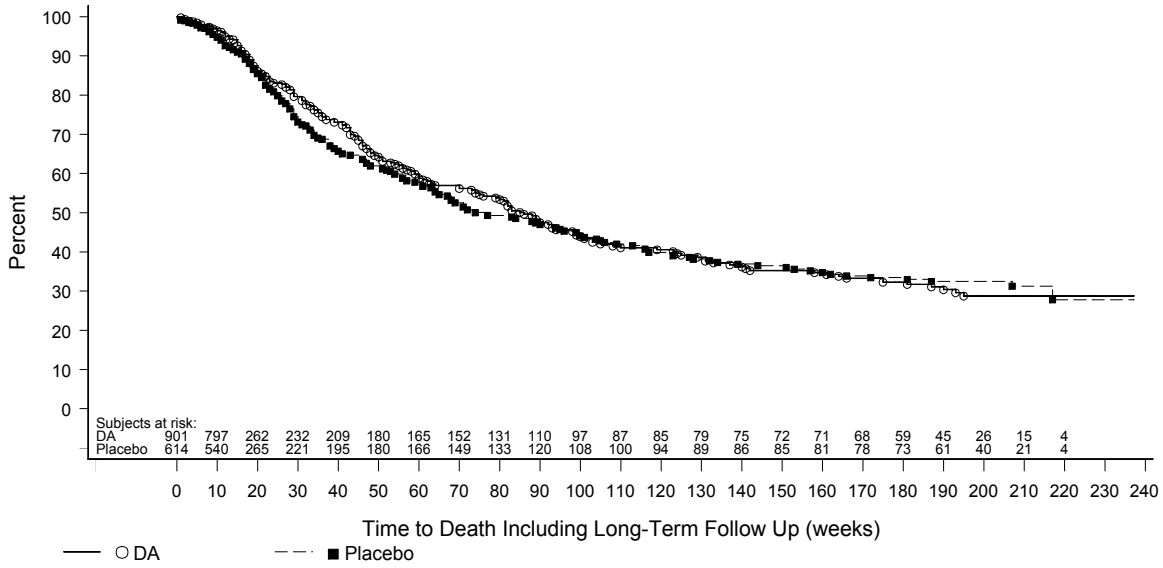
4.2.3 Survival

Patient-level and Study-level Meta-analyses

Kaplan-Meier plots for time to death (including follow-up) for the patient-level analysis of the placebo-controlled trials are provided by week in Figure 2. The curves for darbepoetin alfa and placebo are nearly overlapping during the study period. Similar results were observed for deaths on study (data not shown).

Figure 3 includes the study-level meta-analysis of on-study death for the placebo-controlled studies. The Peto odds ratio was 1.12, with a CI that spanned 1 (CI: 0.748, 1.681).

Figure 2. Kaplan-Meier Curve of Time to Death Including Long-term Follow-up (Placebo-controlled CIA Studies, Randomized Group)



The median time (95% CI) to death including long-term follow up in weeks was 86 (74, 100
) for DA and 77 (64, 100) for Placebo.
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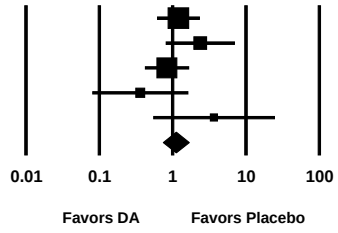
Figure 3. Analysis of Death – Placebo-controlled CIA Studies

Study name	study type	Statistics for each study					Peto odds ratio and 95% CI
		Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value	
CT_980297	1 pc cont	1.200	0.623	2.312	0.545	0.586	
CT_20000161	1 pc cont	2.379	0.818	6.924	1.591	0.112	
CT_20030232	1 pc cont	0.836	0.425	1.645	-0.518	0.604	
CT_980291 Q3W1	pc cont	0.362	0.081	1.614	-1.332	0.183	
CT_980291 Q4W1	pc cont	3.663	0.551	24.331	1.344	0.179	
		1.122	0.748	1.681	0.556	0.578	



Meta Analysis of Reported Deaths by Randomized Treatment Group

Note: no deaths occurred in Study 990114

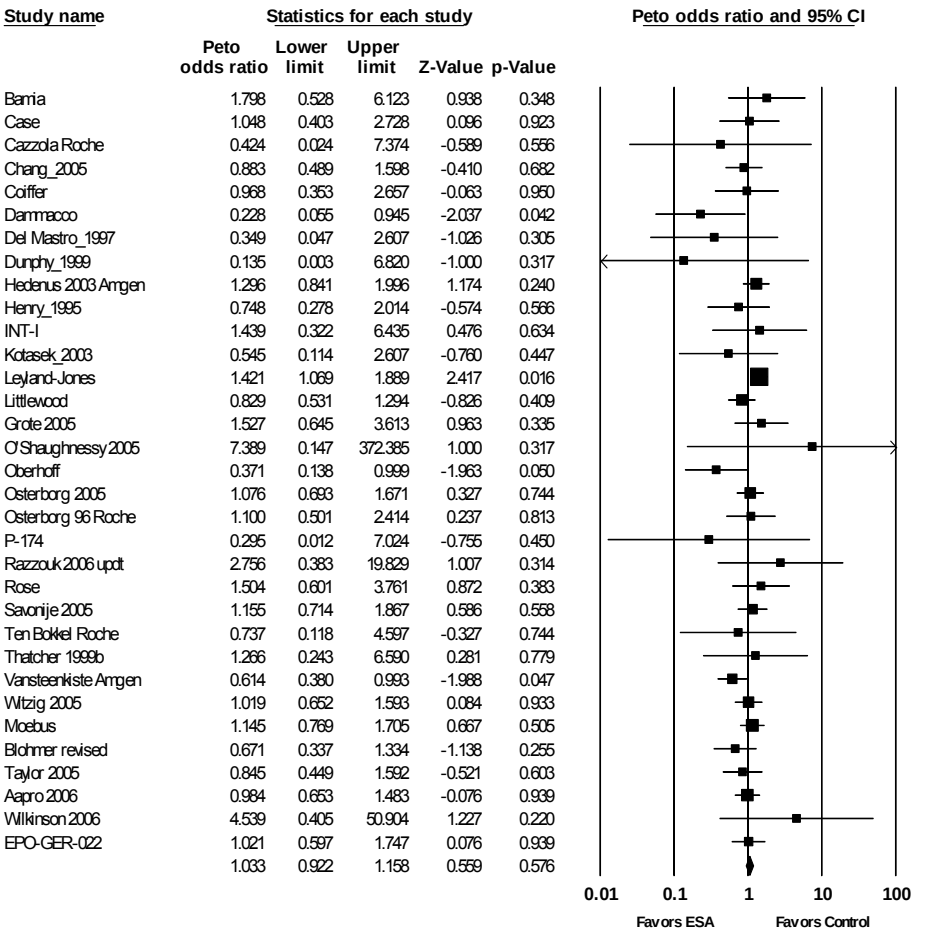
Updated Cochrane Analysis of Survival in Chemotherapy-induced Anemia

The updated Cochrane Collaborative meta-analysis for death is provided in Figure 4 for subjects with CIA. Overall, this analysis includes data from over 8500 patients participating in 35 studies, and represents the most comprehensive summary of such data compiled to date. This analysis updates data for 1 study (Savonije et al, 2005) and

adds data for 6 studies (Aapro et al, 2006 [the BRAVE trial]; Blohmer et al, 2004; Taylor et al, 2005, Wilkinson, 2006; EPO-GER-022; and the Möbus study). Data for Razzouk et al was updated based on a 2006 publication and reclassified as a CIA study (previously categorized as an "unclear" patient population). The study previously identified as N93 004 is now referenced as Grote (2005; data remained unchanged).

In subjects receiving ESAs for CIA, there was a neutral impact on survival. The Peto odds ratio was 1.03 (95% CI: 0.922, 1.158). The sensitivity analysis using the Mantel-Haenszel risk ratio as the statistic rather than the Peto odds ratio was 1.02 (95% CI: 0.958, 1.079; data not shown).

Figure 4. Deaths – Chemotherapy-induced Anemia (Peto Odds Ratio)



Updated Cochrane Collaborative Meta Analysis Using Peto OR

Long-term Survival Data

Amgen has completed two large, phase 3 studies in subjects with CIA that collected information on long-term survival and disease progression (Studies 980297 and 20000161). These studies were conducted in subjects with relatively uniform tumor types: lung cancer in Study 980297, and lymphoproliferative malignancy in Study 20000161. Results of these studies were presented at the 2004 ODAC meeting, including final data for Study 980297 and interim data for Study 20000161. In this section, the final data for Study 20000161 are provided, and the previously presented data for Study 980297 are also summarized for completeness.

4.2.3.1 Lung Cancer: Study 980297

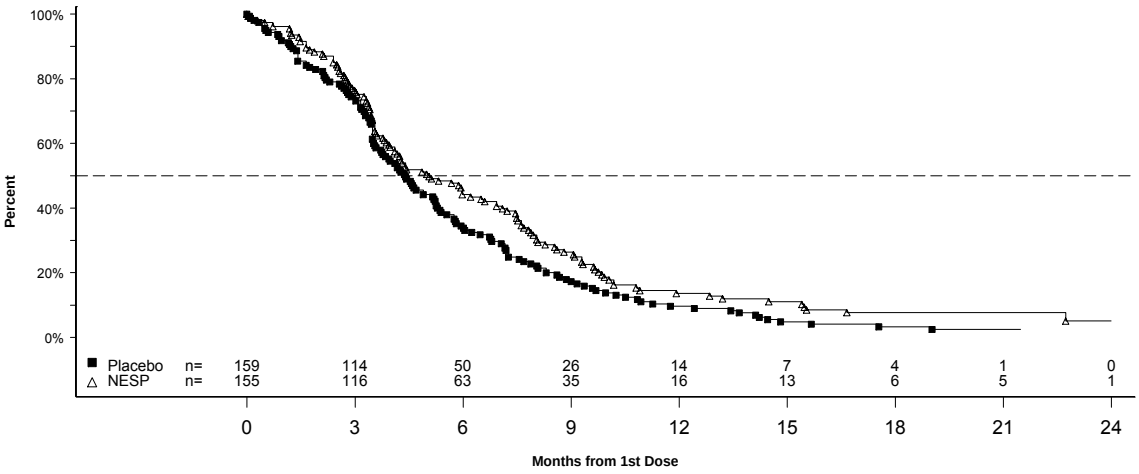
Study 980297 was a multicenter, double-blind, placebo-controlled study to evaluate the effects of darbepoetin alfa at a dose of 2.25 μg/kg once weekly on anemia endpoints in subjects with both non-small cell lung cancer and small cell lung cancer receiving platinum-containing chemotherapy (Vansteenkiste et al, 2002). A total of 314 anemic subjects (hemoglobin concentration ≤ 11.0 g/dL) were randomly assigned and received either darbepoetin alfa or placebo administered weekly as a SC injection for 12 weeks, followed by a 4-week observation period.

Demographic characteristics and baseline disease characteristics in Study 980297 were similar between the darbepoetin alfa and placebo groups (Vansteenkiste et al, 2002). All subjects were white, and 72% were men. Most subjects were < 65 years of age, with a mean (SD) age of 61.4 (8.9) years. Twenty-nine percent of subjects had small cell lung cancer and 71% had non-small cell lung cancer. Tumor type and disease stage were similar in the 2 treatment groups. Most subjects had an ECOG performance status of 0 or 1 (84% darbepoetin alfa, 77% placebo).

All 314 subjects in the safety analysis set were included in the analyses of progression-free survival and overall survival. The observation period started on study day 1, the day on which the first dose of study drug was administered. Two hundred nineteen subjects were followed until death. The median follow-up time was 254 days for darbepoetin alfa and 204 days for placebo.

Overall, subjects in the darbepoetin alfa group had a similar risk of disease progression or death relative to subjects in the placebo group (Figure 5 and Figure 6). Median (95% CI) progression-free survival time was 5 (4, 7) months for the darbepoetin alfa group and 4 (4, 5) months for the placebo group. The estimated relative risk (95% CI) of disease progression or death for the darbepoetin alfa group compared with the placebo group was 0.79 (0.62, 1.00). Median (95% CI) overall survival time was 10 (9, 12) months for the darbepoetin alfa group and 8 (7, 9) months for the placebo group. The estimated relative risk (95% CI) of death for the darbepoetin alfa group compared with the placebo group was 0.77 (0.59, 1.01).

Figure 5. Amgen Study 980297: Kaplan-Meier Curve of Progression-free Survival

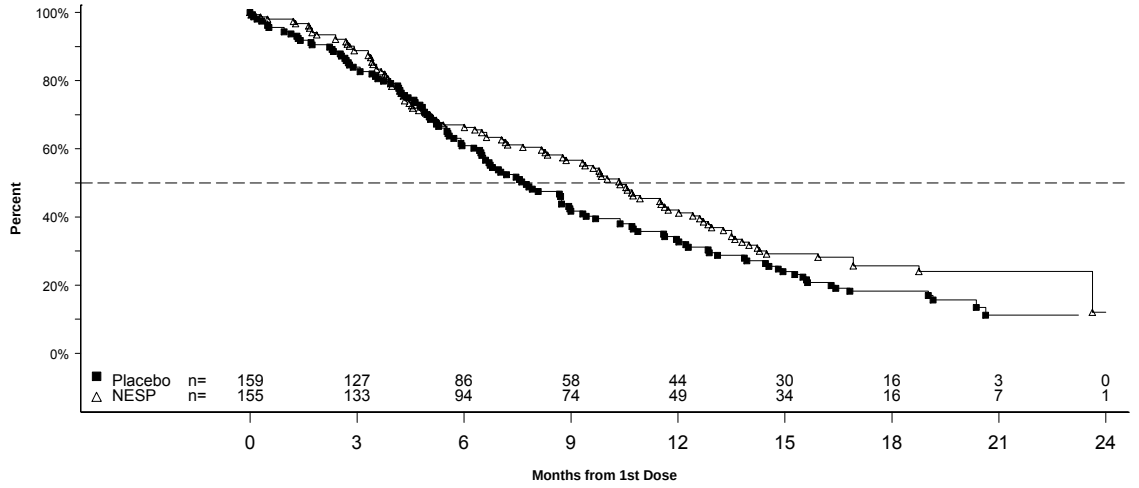


The Kaplan-Meier estimates were truncated at 24 months.

Estimated Relative Risk and 95% Confidence Interval: 0.79 (0.62, 1.00)
The median Progression-Free Survival time in months (95% CI) was 4 (4, 5.3) for Placebo and 5 (4, 6.9) for NESP.

Program: /stat/nesp/onc/meta/nesp20000161x/analysis/interim/fup_oct2003/statfiles/programs/graphs/g_km_fup_RR.sas
Output: /stat/nesp/onc/meta/nesp20000161x/analysis/interim/fup_oct2003/statfiles/programs/graphs/g_km_fup_RR_any04.cgm (Date Generated: 18MAR2004:15:16)

Figure 6. Amgen Study 980297: Kaplan-Meier Curve of Overall Survival



The Kaplan-Meier estimates were truncated at 24 months.

Estimated Relative Risk and 95% Confidence Interval: 0.77 (0.59, 1.01)
The median Overall Survival time in months (95% CI) was 8 (7, 9) for Placebo and 10 (9, 12) for NESP.

Program: /stat/nesp/onc/meta/nesp20000161x/analysis/interim/fup_oct2003/statfiles/programs/graphs/g_km_fup_RR.sas
Output: /stat/nesp/onc/meta/nesp20000161x/analysis/interim/fup_oct2003/statfiles/programs/graphs/g_km_fup_RR_died04.cgm (Date Generated: 18MAR2004:15:16)

Progression and survival endpoints analyzed by histology (non-small cell lung cancer or small cell lung cancer) also revealed no evidence that subjects receiving darbepoetin alfa had an increased risk of disease progression or death relative to subjects in the placebo group (data not shown).

These analyses indicate that darbepoetin alfa was not associated with a lower rate of progression-free survival or overall survival, nor was it associated with an accelerated time to disease progression or death, compared with placebo in this patient population.

4.2.3.2 Lymphoproliferative Malignancy: Study 20000161

Interim data for Study 20000161 were presented at the 2004 ODAC meeting; final results are summarized in this section. Study 20000161 (Hedenus et al, 2003) was a randomized, double-blind, placebo-controlled study in which 344 anemic subjects (hemoglobin concentration $\leq$ 11.0 g/dL) with lymphoid malignancy and CIA received darbepoetin alfa 2.25 μg/kg once weekly or placebo as a SC injection for 12 weeks. No restrictions on prior chemotherapy were included in this study, and subjects were allowed to enter the study at any point during their course of therapy. Randomization was stratified by malignancy type (myeloma versus lymphoma) and previous chemotherapy (heavily pretreated [$\geq$ 2 lines of chemotherapy or 1 line of chemotherapy and a stem-cell transplant] versus not heavily pretreated), which are factors known to affect the severity of anemia or response to ESAs.

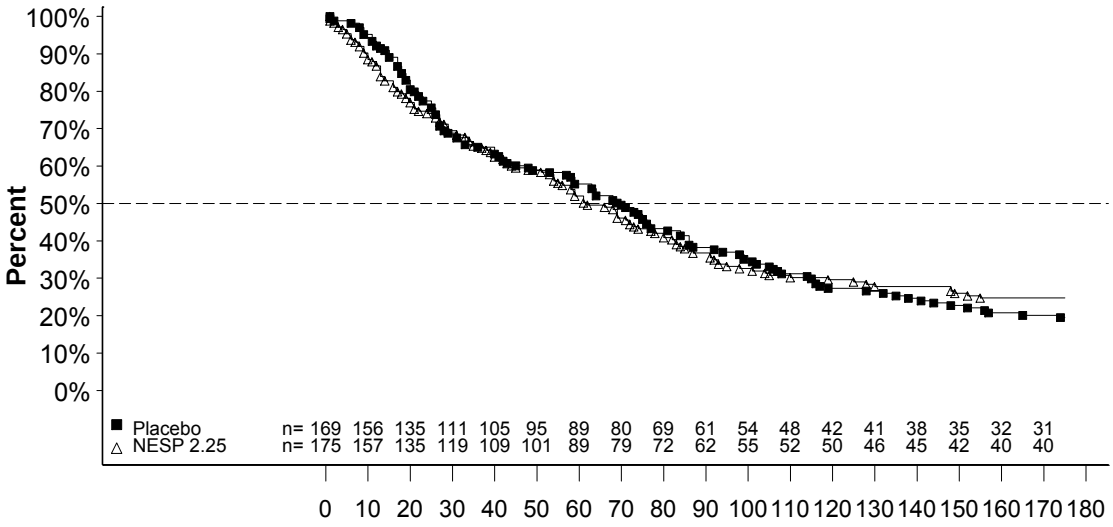
Demographic characteristics for subjects in Study 20000161 were similar between the darbepoetin alfa and placebo groups (Hedenus et al, 2003). Most subjects were white (98%), and 48% were men. Most subjects were $\geq$ 65 years of age, with a mean (SD) age of 64.7 (13.0) years. Overall, 24% of the subjects had non-Hodgkin's lymphoma (NHL), 50% had myeloma, and 16% had chronic lymphocytic leukemia. At the time of initial diagnosis, an imbalance between treatment groups in the percentage of subjects with chronic lymphocytic leukemia (CLL) who had stage C disease (5/8 subjects [63%] in the darbepoetin alfa group, 3/7 subjects [43%] in the placebo group) was observed within the heavily pretreated stratum. However, the small number of subjects makes it difficult to evaluate the potential effect of this difference.

All 344 subjects who received at least 1 dose of investigational product were included in the analyses of survival and progression-free survival. One hundred eighty-three

subjects were followed until death. The median follow-up period was 25 months for darbepoetin alfa and 33 months for placebo.

Subjects in the darbepoetin alfa group had a similar risk of disease progression or death relative to subjects in the placebo group (Figure 7). Median (95% CI) progression-free survival time was 62 (54, 77) weeks for the darbepoetin alfa group and 70 (58, 84) weeks for the placebo group. The estimated relative risk (95% CI) of disease progression or death for the darbepoetin alfa group compared with the placebo group was 1.01 (0.79, 1.29).

Figure 7. Amgen Study 20000161: Kaplan-Meier Curve of Time to Disease Progression or Death Updated for Final Follow-up Assessment (4th April 2005) (Safety Analysis Set)



The Kaplan-Meier curve was truncated at 175 weeks, but the estimates (and 95% CI) were calculated using all available data.

The hazard ratio (95% CI), adjusting for Malignancy Type, Previous Chemotherapy and Region, of NESP 2.25 to Placebo was 1.01 (0.79, 1.29).

The 50th percentile time (95% CI) to Disease Progression or Death was 70 (58, 84) for Placebo and 62 (54, 77) for NESP 2.25.

NESP dose is in units of µg/kg/wk

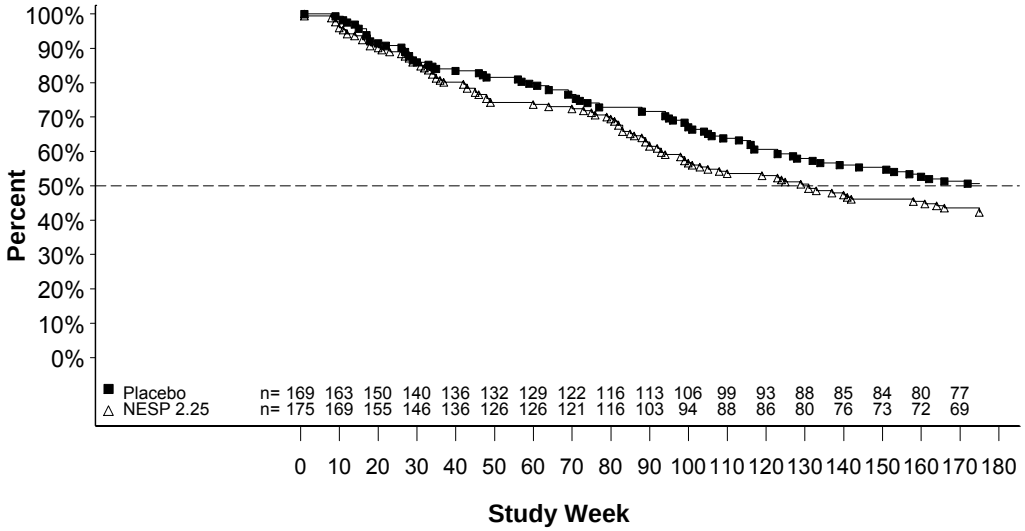
Program: /statistics/nesp/onc/nesp20000161/analysis/interim/final/statfiles/programs/graphs /g_km_fup_nm.sas

Output: g_km_fup_nm_any.cgm (Date Generated: 06DEC2005:11:39)

The Kaplan-Meier curve of time to death is provided in Figure 8. Median (95% CI) overall survival time was 131 (99, 175) weeks for the darbepoetin alfa group and 181 (132, NE) weeks for the placebo group. The estimated relative risk (95% CI) of

death for the darbepoetin alfa group compared with the placebo group was 1.36 (1.02, 1.82).

Figure 8. Amgen Study 20000161: Kaplan-Meier Curve of Time to Death Updated for Final Follow-up Assessment (4th April 2005) (Safety Analysis Set)



The Kaplan-Meier curve was truncated at 175 weeks, but the estimates (and 95% CI) were calculated using all available data.

The hazard ratio (95% CI), adjusting for Malignancy Type, Previous Chemotherapy and Region, of NESP 2.25 to Placebo was 1.36 (1.02, 1.82).

The 50th percentile time (95% CI) to Death was 181 (132, NE) for Placebo and 131 (99, 175) for NESP 2.25.

NESP dose is in units of µg/kg/wk

NE = not estimable

Program: /statistics/nesp/onc/nesp20000161/analysis/interim/final/statfiles/programs/graphs /g_km_fup_nm.sas

Output: g_km_fup_nm_died.cgm (Date Generated: 06DEC2005:11:39)

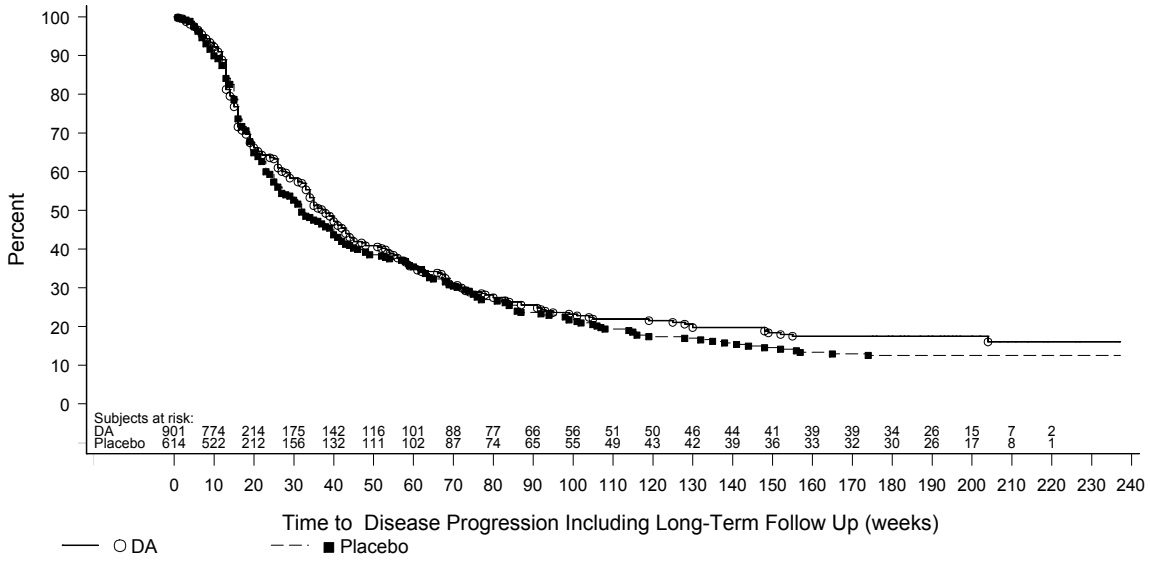
Further analysis of the strata defined by malignancy type and previous chemotherapy indicated that the increased risk in subjects receiving darbepoetin alfa was limited to the heavily pretreated strata (ie, subjects who received ≥ 2 previous lines of chemotherapy or 1 line of chemotherapy and a stem-cell transplant). Because of the small number of subjects in the heavily pretreated myeloma and lymphoma strata, and because the study was not designed to evaluate long-term survival or disease outcomes and was not stratified for relevant prognostic factors, it is difficult to draw any firm conclusions from the data in this study alone.

4.2.4 Disease Progression and Progression-free Survival

As noted in Section 4.1.2, the collection of disease progression information was not an objective of the darbepoetin alfa oncology studies included in this analysis. Therefore, disease progression was identified if the reason for ending study drug or the study was reported as "disease progression" or if the end of study disease status was determined to be "progressive disease" based on the investigator's evaluation.

Kaplan-Meier plots for disease progression in the patient-level pooled analysis of placebo-controlled CIA studies are provided by week for approximately 4.5 years in Figure 9. The curves for darbepoetin alfa and placebo are nearly overlapping during the study period. Results for on-study disease progression and progression-free survival indicated a similar pattern (data not shown).

Figure 9. Kaplan-Meier Curve of Time to Disease Progression Including Long-term Follow-up (Placebo-controlled CIA Studies, Randomized Group)



The median time (95% CI) to disease progression including long-term follow up in weeks was 38 (34, 43) for DA and 32 (27, 40) for Placebo.
Program: /mastat/nesp/onc/meta/odac/200703/graphs/g_km.sas
Output: f5_001_g_km_disprgyn2_fu_cia_pcbctl.cgm (Date Generated: 31MAR07:16:59:26) Source Data: a_sendpt.sas7bdat

Review of ESAs and Disease Progression

Disease progression and related endpoints, such as progression-free survival or relapse-free survival were not reported consistently or regularly in the studies included in

the Cochrane Collaborative analysis. Some studies only reported the ratio between groups, whereas others reported rates within each group. Therefore, with the data available, a formal meta-analysis could not be completed.

The Cochrane Collaborative analysis did not include results for disease progression or related endpoints in their most recent document. However, available data in the literature were summarized in the AHRQ document. The AHRQ data were updated with data from 6 additional studies where results for disease progression/tumor response or a related endpoint were reported (Möbus; Blohmer; Wilkinson; Aapro; Strauss; Leyland-Jones) and summarized below; data for studies using radiotherapy only were excluded from this table. The AHRQ report also listed the Vansteenkiste study, however, disease progression was not a formal endpoint in this study so it was not included in the table below. Finally, the GELA (Delarue) study also reported 1-year event-free survival (73% in 63 patients in DA and 70% in 67 patients in the conventional therapy control arm); however, since the GELA study allowed ESA use in the control arm, this study would have been excluded using the Cochrane analysis criteria and is also excluded from the table below.

Table 4. Disease Progression and Related Endpoints in CIA Studies

Study	Therapy Type	Response Measured	Outcome ESA	Outcome Control
Grote	CIA (SCLC)	PD	8/109 (7%)	9/115 (8%)
Möbus	CIA (breast)	5-year DFS (p=0.89)	72% (n=333 EA)	71% (n=325)
Blohmer	CIA (cervical cancer)	RFS	19/128 (15%)	31/129 (24%)
Wilkinson	CIA (Ovarian)	PD	13/114 (11%)	1/59 (2%)
Aapro (BRAVE)	CIA (Metastatic breast)	PFS	Only HR provided (EA, n =231; control, n = 232) 1.07 (0.89-1.3)	
Strauss	CIA (cervical)	PD	RR 1.08 (0.62-1.87) (EA, n = 34; control, n =40)	
Leyland Jones	(CIA) breast	PD (end of 1st line ctx)	125/469 (27%)	123/470 (26%)
		12-month PD	41% (KM)	43% (KM)
		12-month PFS (p=0.98)	RR: 1.0	
		PD (final assessment)	195/469 (42%)	216/470 (46%)

PD = disease progression; PFS = progression-free survival; DFS = disease-free survival; RFS = relapse-free

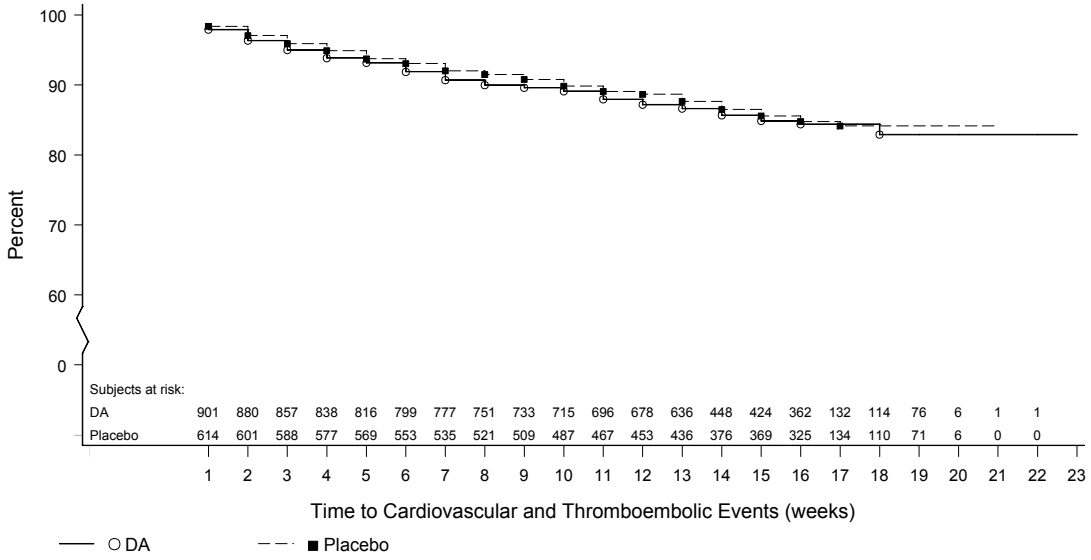
4.2.5 Cardiovascular and Thromboembolic Events

The relationship between thrombosis and cancer is well established; an abundance of research has demonstrated that patients with cancer are at a higher risk for thrombotic events relative to individuals without cancer. In the general population, the risk of deep vein thrombosis or pulmonary embolism is reported to be approximately 117/100,000 (Silverstein et al, 1998). For patients with cancer, the risk is estimated to be approximately 4 times greater, and for those receiving chemotherapy the risk could be 6 times greater for experiencing a TVE (Heit et al, 2000). These findings are supported by a more recent population-based case-control study of 3,220 consecutive patients with a first episode of deep vein thrombosis or pulmonary embolism; the overall risk of venous thrombosis was increased 7-fold in patients with malignancy versus individuals without malignancy (Blom et al, 2005).

In addition, there are many confounding factors that may increase the risk of TVEs in the cancer population, including treatment with chemotherapy, treatment with ESAs, use of intravenous catheters, and periods of immobilization.

Time to first cardiovascular or thromboembolic event is shown in Figure 10 for the patient-level analysis. The Kaplan-Meier curves essentially overlap during the entire study period. The study-level combined analyses of these same studies are provided in Figure 11. The Peto odds ratio for CV/TE events was 1.17 (95% CI: 0.859, 1.585).

Figure 10. Kaplan-Meier Curve of Time to Cardiovascular and Thromboembolic Events (Placebo-controlled CIA Studies, Randomized Group)



The median time (95% CI) to cardiovascular and thromboembolic events in weeks was NE for DA and NE for Placebo.
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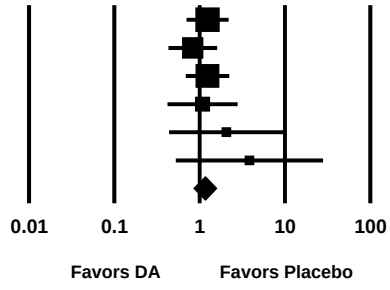
Figure 11. Analysis of Cardiovascular and Thrombotic Events – Placebo-controlled CIA Studies

Study name	study type	Statistics for each study					Peto odds ratio and 95% CI
		Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value	
CT_980297	1 plbo cntl	1.238	0.713	2.150	0.760	0.447	
CT_20000161	1 plbo cntl	0.831	0.439	1.572	-0.570	0.569	
CT_20030232	1 plbo cntl	1.236	0.699	2.185	0.727	0.467	
CT_980291 Q3W1	plbo cntl	1.082	0.427	2.743	0.166	0.868	
CT_980291 Q4W1	plbo cntl	2.062	0.446	9.535	0.926	0.354	
CT_990114	1 plbo cntl	3.838	0.536	27.473	1.339	0.181	
		1.167	0.859	1.585	0.987	0.323	

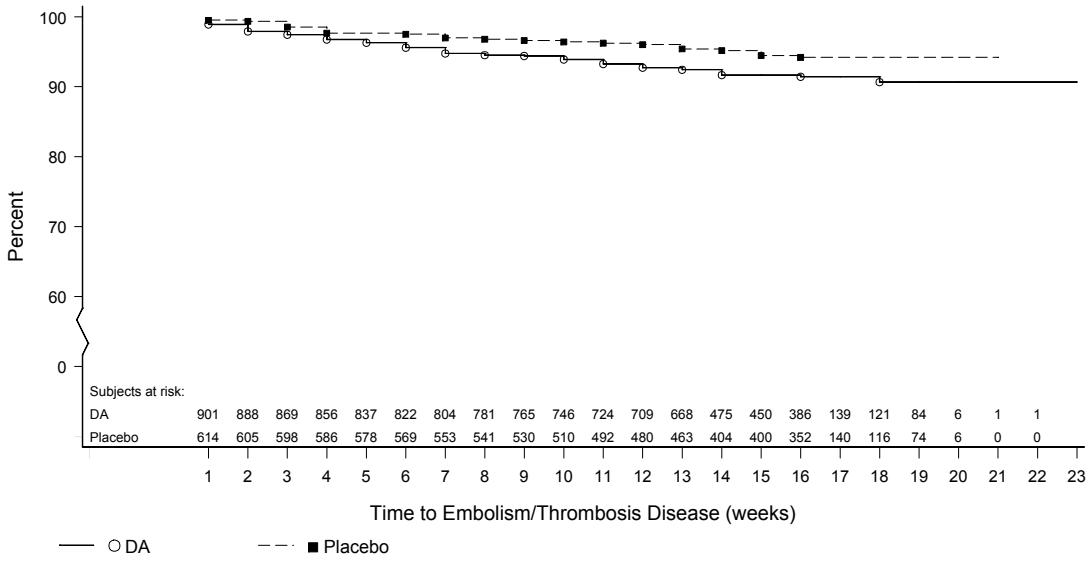


Meta Analysis of Cardiovascular and Thromboembolic Events by Randomized Treatment Group

Time to first embolism or thrombosis event, a subcategory of CV/TE events, is shown in Figure 12 for the patient-level analysis. The Kaplan-Meier curves show an early separation of curves that remains nearly proportional during the study period. The corresponding study-level combined analyses are provided in Figure 13. The Peto odds

ratio for embolism or thrombosis events for the study-level analysis was 1.52 (95% CI: 0.987, 2.343). However, as noted previously, the incidence seen across all studies of 7.8% in the darbepoetin alfa group and 5% in the placebo group is consistent with rates previously reported.

Figure 12. Kaplan-Meier Curve of Time to Embolism/Thrombosis Disease (Placebo-controlled CIA Studies, Randomized Group)



The median time (95% CI) to embolism/thrombosis disease in weeks was NE for DA and NE for Placebo.
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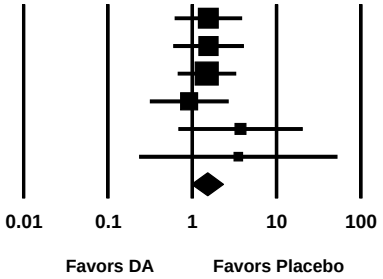
Output: f1_001_g_km_emthryn_cia_pcbctl.cgm (Date Generated: 31MAR07:16:58:33) Source Data: a_sendpt.sas7bdat

Figure 13. Analysis of Embolism/Thrombosis – Placebo-controlled CIA Studies

Study name	study type	Statistics for each study					Peto odds ratio and 95% CI
		Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value	
CT_980297	1 pc cont	1.552	0.628	3.834	0.952	0.341	
CT_20000161	1 pc cont	1.558	0.604	4.019	0.917	0.359	
CT_20030232	1 pc cont	1.488	0.681	3.249	0.996	0.319	
CT_980291 Q3W	1 pc cont	0.919	0.318	2.654	-0.157	0.876	
CT_980291 Q4W	1 pc cont	3.728	0.694	20.028	1.534	0.125	
CT_990114	1 pc cont	3.519	0.238	51.961	0.916	0.360	
		1.521	0.987	2.343	1.902	0.057	

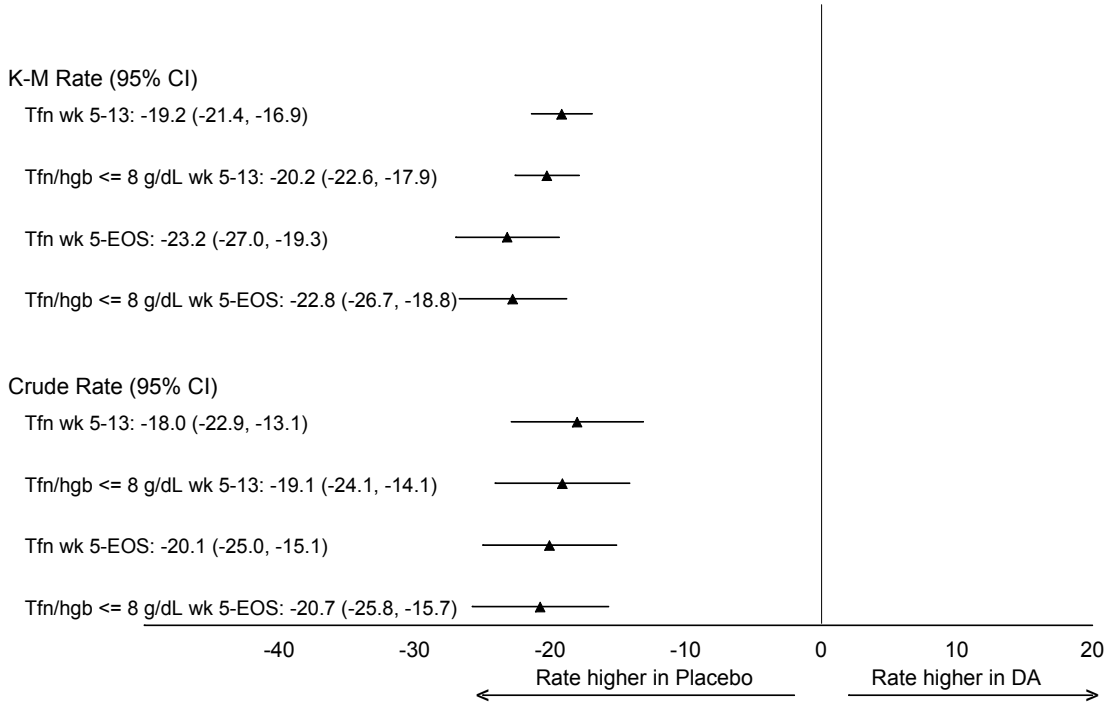


Meta Analysis of Embolism/Thrombosis by Randomized Treatment Group

4.2.6 Clinical Evidence of Benefit – Transfusions and Hematopoietic Response

To allow an evaluation of the risk/benefit of darbepoetin alfa based on currently available data, patient-level analyses of RBC transfusions and hematopoietic response (defined as an increase in hemoglobin of 2.0 g/dL or a value of 12.0 g/dL in the absence of RBC transfusions in the previous 28 days) were also conducted. Significant reductions in the risk of transfusions from week 5 onward were observed for darbepoetin alfa relative to placebo (Figure 14). Sensitivity analyses evaluating the occurrence of either a transfusion or a hemoglobin concentration ≤ 8.0 g/dL also demonstrated a significant benefit for darbepoetin alfa relative to placebo (data not shown). Similarly, subjects receiving darbepoetin alfa were more likely to achieve a hematopoietic response than subjects receiving placebo (Figure 15).

Figure 14. Difference in Transfusion Rates: Week 5 Onward (Placebo-controlled CIA Studies, Randomized Group)



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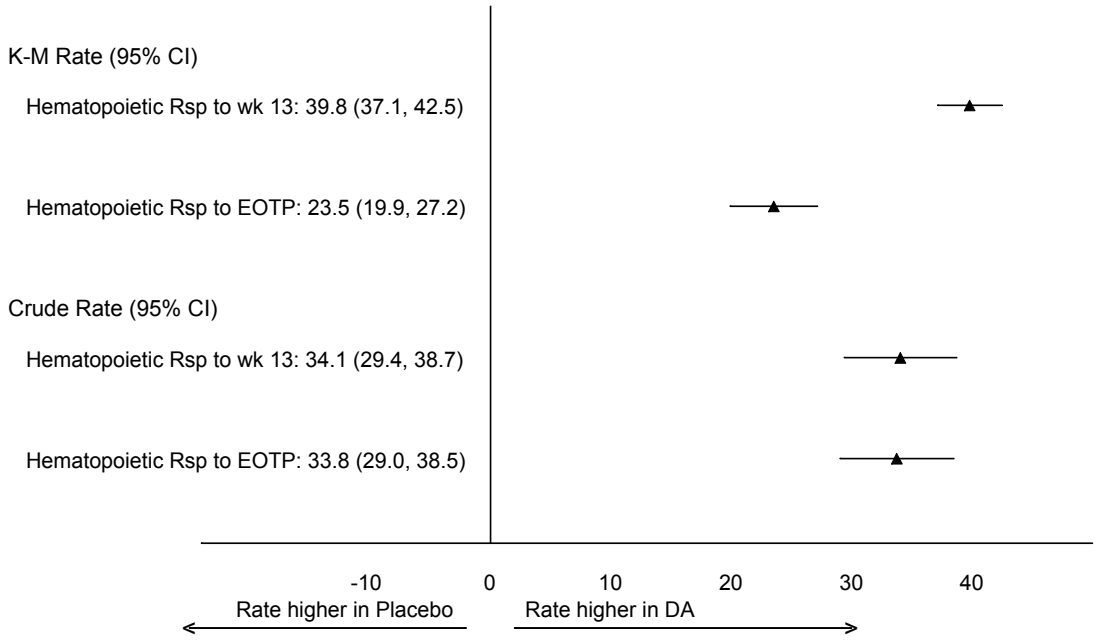
Output: g_2_tfn_CIA.cgm (Date Generated: 02APR07:12:18) Source Data: tx_h.sas7bdat

Figure 15. Difference in Hematopoietic Response Rates (Placebo-controlled CIA Studies, Randomized Group)



Program: /mastat/nesp/onc/meta/odac/200703/graphs/g_fplot.sas
Output: g_3_hgb_CIA.cgm (Date Generated: 02APR07:12:19) Source Data: tx_h.sas7bdat

4.2.7 Predicting Risk in Patients Receiving ESAs for the Treatment of Chemotherapy-induced Anemia

4.2.7.1 Analysis of Potential Risk Factors

The pooled patient-level data analysis of placebo-controlled studies included an evaluation of factors that could have influenced death, disease progression, or CV/TE adverse events (see Section 4.1.3 for description). The hazards ratio (HR) using a Cox-proportional hazards regression including only treatment group in the model was compared to a single "full" risk-factor adjusted HR which included treatment group as well as all potential risk factors (Table 5).

Table 5. Hazards Ratio[a] for Reduced and Full Models (Subjects in Placebo-Controlled, Chemotherapy-Induced Anemia Studies by Treatment Group)

	Variables in Model[b]			
	Treatment Group Only		Treatment Group + Covariates	
	HR	95% CI	HR	95% CI
Death				
On-study	1.14	0.76, 1.70	1.17	0.72, 1.89
Including follow-up	0.99	0.82, 1.19	1.06	0.86, 1.31
Disease Progression				
On-study	0.87	0.70, 1.09	0.81	0.62, 1.06
Including follow-up	0.85	0.73, 0.99	0.86	0.72, 1.03
Progression-free Survival				
On-study	0.91	0.74, 1.12	0.87	0.68, 1.12
Including follow-up	0.88	0.76, 1.01	0.89	0.76, 1.05
Cardiovascular or Thrombotic Events	1.15	0.86, 1.52	1.20	0.85, 1.70
Embolism or Thrombosis	1.50	0.97, 2.33	1.79	1.01. 3.17

[a] Hazards ratio are calculated as the hazard of an event in the darbepoetin alfa group compared to the placebo group (treatment effect)
[b]Study protocol included as a stratification factor for all models
HR is the treatment effect in both models

Adapted from: Adapted from Output: died_C_p died_C_pfull died2_C_p died2_Cpfull dis_C_p dis_C_pfull dis2_C_pl dis2_C_pfull pfsy_C_pl pfsy_C_pfull pfs2_C_pfull pfs2_C_pfull cart_C_p cart_C_pfull emth_C_pf emth_C_pfull (Date Generated: 31MAR07:16:31-16_38)

Visual comparison of the unadjusted and adjusted HRs suggests the effect of treatment relative to placebo with respect to the risk of death, disease progression, and CV/TE was not substantially changed by the inclusion of these baseline risk factors (Table 5) even if some of the risk factors were statistically significant in the Cox proportional hazards model (data not shown). Sex, weight, ECOG status, baseline FACT-F score, disease stage, baseline hemoglobin, and endogenous EPO level were statistically significantly associated with an impact on on-study disease progression or survival outcomes, including death during follow-up. ECOG, FACT-F, and disease stage are recognized as significant predictors of survival outcomes in oncology patients. The effect of sex on these events was unexpected, and whether this effect was related to gender differences alone or gender-related differences in tumor types is not clear. The effect of weight may be related to a lower weight (eg, due to cachexia) in subjects with more advanced

disease. The association of lower baseline hemoglobin or higher baseline EPO level and poorer survival outcomes suggests a potential link between known predictors of treatment response and survival, which is investigated in exploratory analyses below (Association Between Hemoglobin and Safety Outcomes).

For CV/TE events, associated risk factors included age (a known risk factor for cardiovascular disease), higher baseline EPO (a marker of poor response), and a previous history of thrombosis.

For thrombosis/embolism, the only significant risk factor identified was a previous history of thrombosis (a recognized risk factor for recurrent thrombosis).

A separate analysis of transfusions as a time-dependent covariate was also done. For this analysis, ever having a transfusion was the time-dependent covariate and death, disease progression, and death or disease progression were three separate outcome variables of interest. This analysis was done separately for placebo and darbepoetin alfa patients and included study protocol as a stratification variable. Transfusions on-study were significantly associated with an increased risk of death, disease progression, and reduced progression-free survival for both the darbepoetin alfa and placebo groups (Table 6). Given that transfusion requirements may be higher in patients who have a poorer health status and therefore have a higher risk of death or progression, this analysis may potentially be confounded.

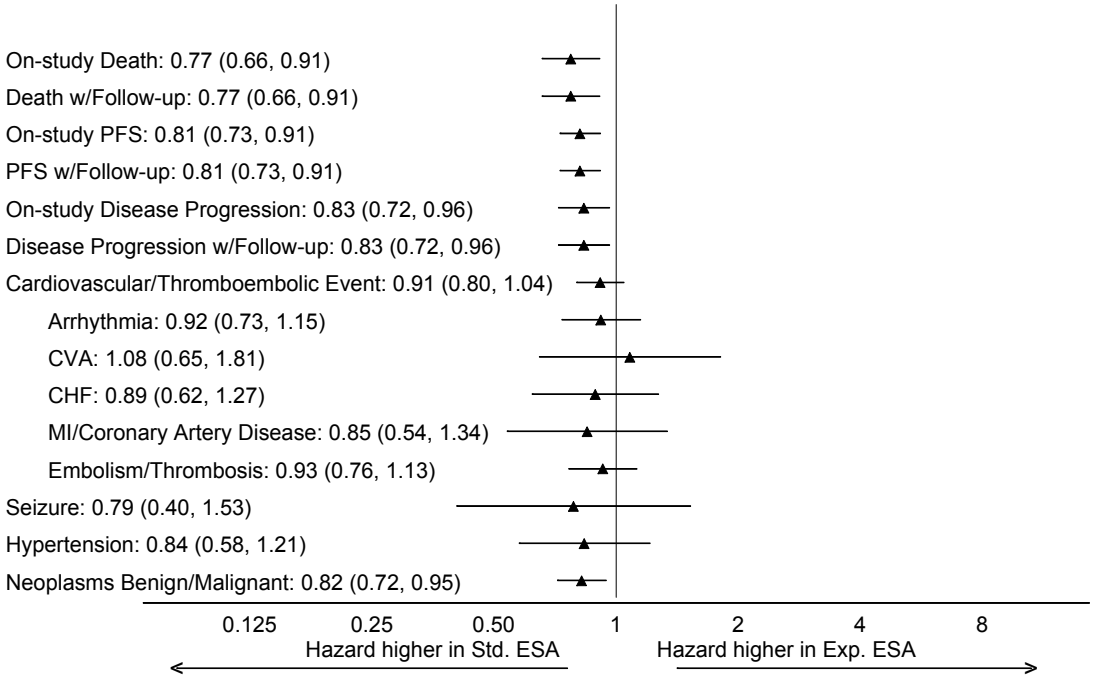
Table 6. Hazards Ratio for Transfusion as a Time Dependent Covariate (Subjects in Placebo-Controlled, Chemotherapy-Induced Anemia Studies by Treatment Group)

	Time Dependent Covariate Analyzed			
	Darbepoetin alfa		Placebo	
	HR	95% CI	HR	95% CI
Death				
On-study	2.43	1.36, 4.32	4.06	1.97, 8.38
Including follow-up	1.55	1.16, 2.06	1.87	1.42, 2.46
Disease Progression				
On-study	1.94	1.48, 2.54	2.46	1.78, 3.38
Including follow-up	1.52	1.22, 1.89	1.58	1.27, 1.97
Progression-free Survival				
On-study	2.06	1.59, 2.67	2.68	1.95, 3.69
Including follow-up	1.57	1.27, 1.93	1.73	1.40. 2.14
Cardiovascular or Thrombotic Events	2.07	1.38, 3.11	1.59	0.99, 2.57
Embolism or Thrombosis	1.35	0.76, 2.39	1.18	0.50. 2.77

4.2.7.2 Effect of Dose on Safety Outcomes

To evaluate the effect of dose on the risk of adverse events of interest, a pooled patient-level analysis of these events was done for studies where a higher dose of darbepoetin alfa was given (n = 2776) relative to a control in which an appropriate (that is, consistent with the label) dose of the same or another ESA was given (n = 2609). In this analysis, the higher-dose darbepoetin alfa group did not show an increased hazard relative to the standard dose group (Figure 16). In particular, death and disease progression appeared to be reported less frequently in the higher-dose group than in the standard ESA group. For CV/TE events, a HR of 0.91 (95% CI: 0.80, 1.04) was seen in the higher-dose group compared to the standard ESA group. Results for the subcategory of TEs showed similar results, with a HR of 0.93 (95% CI: 0.76, 1.13).

Figure 16. Adverse Event Hazard Ratios
(Active-controlled CIA Studies, Randomized Group)



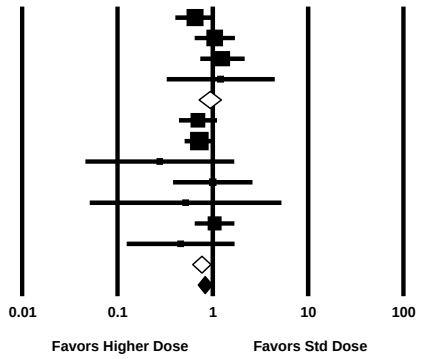
Immune System Disorder and Pure Red Cell Aplasia have been removed from this figure.

Program: /mastat/nesp/onc/meta/odac/200703/graphs/g_hazard_actgroup.sas
Output: (Date Generated: 01APR07:17:38) Source Data: act_h.sas7bdat

Study-level combined analyses of active-controlled studies are provided in Figure 17, Figure 18, and Figure 19. In these analyses, the control group was defined as a standard dose of an ESA – either 40,000 U QW or 150 U/kg three times a week for epoetin alfa or 2.25 μg/kg QW for darbepoetin alfa. The test doses were either higher doses given initially followed by a standard (or lower) dose ("front-loaded", designated as "fl") or doses which were given less frequently but at a higher dosage per injection ("less frequent dosing", designated as "lfd"). The odds of death (Figure 17), CV/TE adverse events (Figure 18), and TE events (Figure 19) were not higher in the test groups compared to the standard dose groups.

Figure 17. Analysis of Deaths – Active-controlled CIA Studies by Study Type

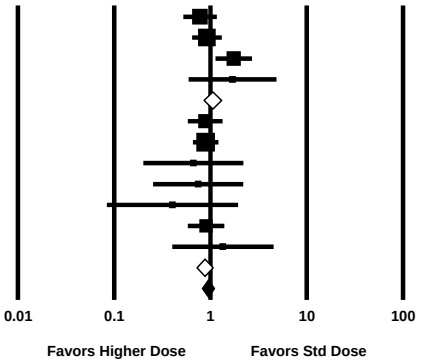
Group by Subgroup within study	Study name	study type	Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value
2 FL	CT_20020118	2 FL	0.652	0.413	1.030	-1.835	0.067
2 FL	CT_20010101	2 FL	1.049	0.657	1.676	0.201	0.841
2 FL	CT_20020139	2 FL	1.264	0.754	2.120	0.888	0.374
2 FL	CT_20000174	2 FL	1.210	0.334	4.387	0.291	0.771
2 FL			0.941	0.718	1.233	-0.444	0.657
3 LFD	CT_20030231	3 LFD	0.698	0.449	1.086	-1.593	0.111
3 LFD	CT_20030125	3 LFD	0.724	0.515	1.018	-1.859	0.063
3 LFD	CT_20020152	3 LFD	0.278	0.047	1.648	-1.410	0.159
3 LFD	CT_20020165	3 LFD	1.000	0.391	2.558	0.000	1.000
3 LFD	CT_20020166	3 LFD	0.518	0.052	5.157	-0.561	0.575
3 LFD	CT_20040262	3 LFD	1.045	0.657	1.661	0.186	0.852
3 LFD	CT_980290 Q2W	3 LFD	0.460	0.127	1.667	-1.182	0.237
3 LFD			0.769	0.617	0.959	-2.336	0.019
Overall			0.833	0.703	0.989	-2.092	0.036



Meta Analysis of Reported Deaths by Randomized Treatment Group

Figure 18. Analysis of Cardiovascular and Thrombotic Events – Active-Controlled CIA Studies

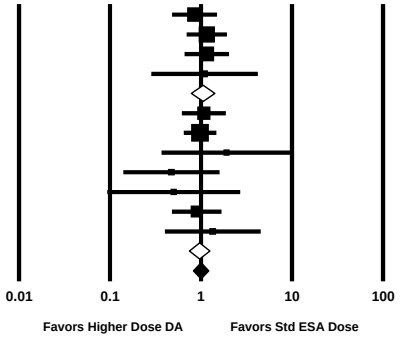
Group by Subgroup within study	Study name	study type	Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value
2 fl	CT_20020118	2 fl	0.779	0.531	1.143	-1.276	0.202
2 fl	CT_20010101	2 fl	0.919	0.656	1.288	-0.489	0.625
2 fl	CT_20020139	2 fl	1.747	1.151	2.653	2.618	0.009
2 fl	CT_20000174	2 fl	1.695	0.604	4.758	1.002	0.317
2 fl			1.058	0.856	1.307	0.519	0.604
3 lfd	CT_20030231	3 lfd	0.881	0.592	1.311	-0.626	0.531
3 lfd	CT_20030125	3 lfd	0.893	0.670	1.190	-0.774	0.439
3 lfd	CT_20020152	3 lfd	0.665	0.204	2.162	-0.678	0.498
3 lfd	CT_20020165	3 lfd	0.746	0.258	2.156	-0.542	0.588
3 lfd	CT_20020166	3 lfd	0.403	0.085	1.903	-1.147	0.251
3 lfd	CT_20040262	3 lfd	0.901	0.591	1.372	-0.486	0.627
3 lfd	CT_980290 Q2W	3 lfd	1.345	0.408	4.439	0.487	0.626
3 lfd			0.878	0.724	1.065	-1.321	0.186
Overall			0.955	0.828	1.102	-0.627	0.531



Meta Analysis of Cardiovascular and Thromboembolic Events by Randomized Treatment Group

Figure 19. Analysis of Embolism/Thrombotic Events – Active-Controlled CIA Studies

Group by Subgroup within study	Study name	study type	Peto odds ratio	Lower limit	Upper limit	Z-Value	p-Value
2 FL	CT_20020118	2 FL	0.849	0.490	1.471	-0.584	0.559
2 FL	CT_20010101	2 FL	1.158	0.710	1.890	0.588	0.556
2 FL	CT_20020139	2 FL	1.157	0.670	1.996	0.524	0.601
2 FL	CT_20000174	2 FL	1.095	0.290	4.140	0.133	0.894
2 FL			1.055	0.785	1.419	0.355	0.723
3 LFD	CT_20030231	3 LFD	1.075	0.628	1.838	0.263	0.793
3 LFD	CT_20030125	3 LFD	0.975	0.658	1.445	-0.128	0.899
3 LFD	CT_20020152	3 LFD	1.911	0.374	9.753	0.779	0.436
3 LFD	CT_20020165	3 LFD	0.473	0.143	1.569	-1.223	0.221
3 LFD	CT_20020166	3 LFD	0.502	0.095	2.649	-0.811	0.417
3 LFD	CT_20040262	3 LFD	0.898	0.489	1.650	-0.347	0.729
3 LFD	CT_980290 Q20	3 LFD	1.345	0.408	4.439	0.487	0.626
3 LFD			0.965	0.744	1.252	-0.267	0.789
Overall			1.003	0.825	1.220	0.034	0.973



Meta Analysis of Embolism/Thrombotic Events by Randomized Treatment Group

4.2.7.3 Association Between Hemoglobin Achieved and Safety Outcomes

An exploratory analysis was done for subjects receiving darbepoetin alfa in placebo-controlled studies to determine if reaching a hemoglobin > 12 g/dL, > 13 g/dL, or having had a rate of rise in hemoglobin in excess of 1 g/dL in a 14-day window in the past is associated with an increased incidence of death, disease progression, CV/TE adverse events, or other adverse events of interest. As these factors could not be determined or predicted at baseline, the impact of each factor was assessed individually as a time-dependent covariate in a Cox proportional hazards model. Since the analysis was limited to only those subjects randomized to receive darbepoetin alfa, neither treatment group nor study was included in the model.

The incidence of on-study death was lower after these hemoglobin events than prior to them: HR = 0.41 (95% CI: 0.20, 0.83) for hemoglobin > 12 g/dL, HR = 0.60 (95% CI: 0.25, 1.45) for a hemoglobin > 13 g/dL, and HR = 0.48 (95% CI: 0.26, 0.89) for a 1-g/dL increase in a 14-day window. A similar pattern was seen when deaths were also identified during a study's follow-up period.

Disease progression and disease progression or death (labeled PFS) also were lower in subjects who had a hemoglobin concentration > 12 g/dL (HR: 0.45 to 0.67), > 13 g/dL (HR: 0.63 to 0.84), or a 1-g/dL increase in a 14-day window (HR: 0.55 to 0.64). The

ability to reach a hemoglobin level > 12 g/dL or > 13 g/dL may identify a subgroup of patients who are healthier.

Although statistical significance was not reached, these hemoglobin factors were also associated with an increase in TE events. The HR associated with having had a hemoglobin > 12 g/dL compared to not reaching this level was 1.66 (95% CI: 0.90, 3.04), and the HR associated with having had a hemoglobin > 13 g/dL compared to not reaching this level was 1.82 (95% CI: 0.86, 3.83) (Table 7). For subjects receiving darbepoetin alfa, the HR associated with having had a 1-g/dL increase in hemoglobin within a 14-day window compared to not having a 1-g/dL increase was 1.67 (95% CI: 0.96, 2.88). The increased risk noted in this analysis is consistent with that previously observed in this subject population. As the ability to reach higher hemoglobin concentrations may be reflective of a better prognosis, these analyses may be confounded with responsiveness to treatment.

Table 7. Hazards Ratio for Time Dependent Covariates (Darbepoetin alfa Subjects in Placebo-Controlled, Chemotherapy-Induced Anemia Studies)

	Time Dependent Covariate Analyzed					
	Hgb[a] > 12 g/dL		Hgb[a] > 13 g/dL		> 1 g/dL Increase in Hgb[a] 14 Days	
	HR	95% CI	HR	95% CI	HR	95% CI
Death						
On-study	0.41	0.20, 0.83	0.60	0.25, 1.45	0.48	0.26, 0.89
Including follow-up	0.57	0.43, 0.75	0.68	0.51, 0.92	0.53	0.40, 0.70
Disease Progression						
On-study	0.45	0.33, 0.60	0.63	0.44, 0.90	0.57	0.43, 0.75
Including follow-up	0.67	0.54, 0.83	0.84	0.67, 1.05	0.64	0.51, 0.79
Disease Progression or Death						
On-study	0.46	0.35, 0.61	0.65	0.46, 0.92	0.55	0.42, 0.72
Including follow-up	0.65	0.53, 0.79	0.81	0.64, 1.01	0.62	0.50, 0.77
Cardiovascular or Thrombotic Events	0.85	0.52, 1.39	1.16	0.62, 2.17	0.89	0.58, 1.37
Embolism or Thrombosis	1.66	0.90, 3.04	1.82	0.86, 3.83	1.67	0.96, 2.88

[a]: *Hemoglobin values within 28 days after a transfusion were not included*

4.3 Assessment of Darbepoetin alfa for the Treatment of Anemia in Active Cancer in the Absence of Chemotherapy or Radiotherapy: Study 20010103

Amgen has conducted 4 clinical studies of darbepoetin alfa in subjects with cancer not receiving chemotherapy or radiotherapy. Initial phase 2 studies of darbepoetin alfa in anemic subjects with cancer who were not receiving chemotherapy or radiotherapy (990111 and 20000219) suggested a favorable risk/benefit profile in this setting. Based on these results, a registrational pathway was proposed for this indication, and a pivotal phase 3 study (Study 20010103) was designed. In support of the proposed indication, the population for this study was originally defined as subjects with anemia due to their cancer or prior treatment who were not planning to receive chemotherapy or radiotherapy. However, after discussions with FDA, the population was modified to exclude subjects who were in complete remission of their neoplastic disease, ie, subjects had to have evidence of an active malignancy.

The study inclusion requirements for subjects with active cancer neither receiving nor planning to receive chemotherapy identify a specific subset of patients with anemia of cancer that are mutually exclusive with those required for a study of CIA, and potentially a more advanced group of cancer patients. That this is the case is suggested by an on-study death rate roughly 2- to 3-fold higher than that seen in either typical CIA studies or the phase 2 studies in AOC.

Because Study 20010103 was designed to evaluate the effect of darbepoetin alfa on RBC transfusions and not survival, deaths were collected on the adverse event case report form or as a reason for study drug/study termination and were not adjudicated. It therefore must be recognized that although deaths in subjects in this advanced stage of disease were likely to be attributed to their malignancy, in this population that is already at a higher risk for thrombotic events, other potential causes such as TVEs cannot be excluded.

In this study, subjects were randomized in a 1:1 ratio to receive darbepoetin alfa 6.75 μg/kg Q4W or placebo for 16 weeks. Randomization was stratified by geographic region, screening hemoglobin, recent RBC transfusion, ECOG score, and tumor type/treatment category. Upon achieving a predefined number of transfusion events (145 subjects with at least 1 RBC transfusion), the randomization allocation ratio was

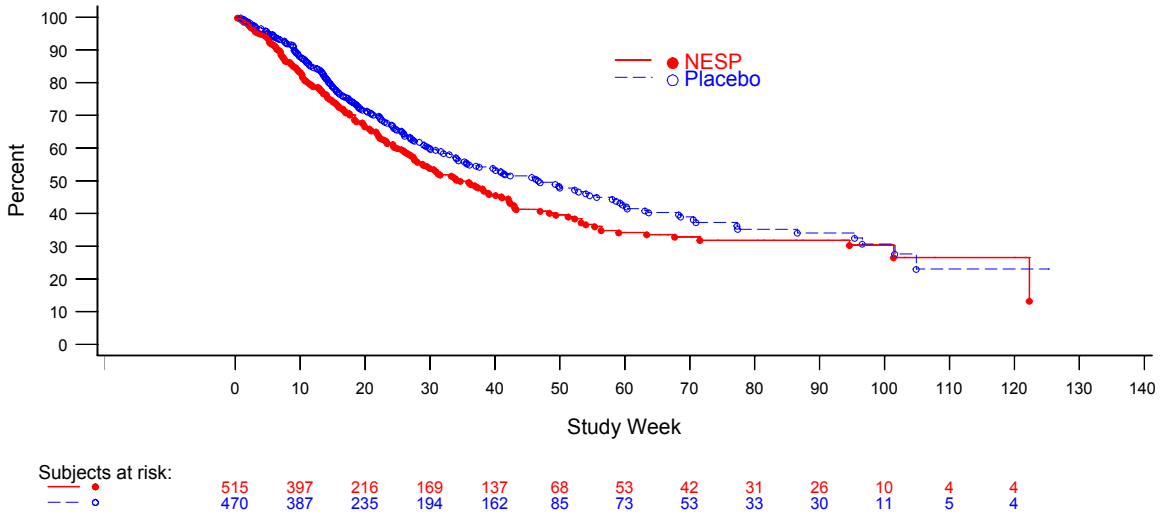
changed from 1:1 to 9:1 (darbepoetin alfa: placebo) until a total of 500 subjects were randomized to the darbepoetin alfa group.

The darbepoetin alfa group had a higher percentage of men (55.3%) than the placebo group (46.8%). Mean age was similar between groups. More subjects in the darbepoetin alfa group than the placebo group had stage III or IV disease at baseline (82.7% versus 80.6%, respectively) or had received prior cytotoxic chemotherapy (74.0% versus 66.0%, respectively). The percentage of subjects considered to have progressive disease at baseline by investigator assessment was 44% in the darbepoetin alfa group and 41% in the placebo group.

This study did not demonstrate the effectiveness of darbepoetin alfa 6.75 µg/kg Q4W in reducing the total occurrence of RBC transfusions during weeks 5 to 17 (Kaplan-Meier percentage of 19.1% for darbepoetin alfa and 24.0% for placebo), with a hazard ratio of 0.85 (95% CI: 0.62, 1.17) ($p = 0.32$). A prespecified sensitivity analysis of the first occurrence of either a red blood cell transfusion or a hemoglobin concentration ≤ 8 g/dL during weeks 5 to 17 yielded a statistically significant difference between treatment groups favoring darbepoetin alfa ($p = 0.02$). In addition, time to first RBC transfusion during weeks 5 to 17 (a secondary efficacy endpoint) was significantly prolonged in subjects receiving darbepoetin alfa relative to those receiving placebo (hazard ratio 0.74; 95% CI 0.55, 0.99, $p = 0.045$). Significant increases in hemoglobin from baseline to the end of the treatment period were noted in the darbepoetin alfa group.

At the time of the first data cutoff for the overall survival analysis, 466 subjects had died either during the study or the long-term follow-up period (250 darbepoetin alfa [48.5%], 216 placebo [45.9%]). The overall Kaplan-Meier curve of time to all deaths by treatment group is presented in Figure 20.

Figure 20. Amgen Study 20010103: Kaplan-Meier Curves of Overall Survival by Treatment (Safety Analysis Set)



NESP= Darbepoetin alfa 6.75 µg/kg Q4W
Percentage with event = 1 - KM estimate by treatment group
Note: Time to death is number of days from the first day of investigational product administration to the date of death .
Program: /stat/nesp/onc/nesp20010103/analysis/exploratory/statfiles/programs/graphs/g_km_death.sas
Output: g_km_death_all_saf.cgm (Date Generated: 27MAR07:14:20:24) Source Data: c_chemo, c_diag, c_disrsp, a_sendpt.sas7bdat

The hazard ratio of time to all deaths in the darbepoetin alfa group relative to the placebo group was 1.29 (95% CI: 1.08, 1.55) (p = 0.006), based on the Cox regression analysis stratified by the factors used at randomization but unadjusted for additional covariates (Table 8). In post-hoc analyses adjusting for stratification factors at randomization, a significant difference in survival remained between the groups; however, hazard ratios and the statistical significance of the treatment difference diminished when the analysis was further adjusted for baseline imbalances. Depending on the specific analysis employed, the hazard ratios of time to all deaths in the darbepoetin alfa group relative to the placebo group ranged from 1.17 to 1.29 (Table 8). Sex, stage IV disease, ECOG performance status, region, baseline hemoglobin level and prior transfusion within 12 weeks of enrollment were all statistically significant in the model adjusting for stratification factors and covariates.

Table 8. Amgen Study 20010103: Stratified Cox Regression Analysis for Time to All Deaths

	Hazard Ratio	95% CI	p-value
Stratified analysis	1.29	1.08, 1.55	0.006
Adjusted for stratification factors at randomization	1.23	1.02, 1.48	0.031
Adjusted for stratification factors and covariates (sex, stage IV disease, prior cytotoxic chemotherapy use and prior radiotherapy use)	1.18	0.98, 1.42	0.082
Adjusted for known prognostic factors at baseline (ECOG, tumor type, tumor stage, FACT-F cutoff at median, hemoglobin)	1.17	0.96, 1.42	0.111

In summary, this study did not meet its primary endpoint of reducing transfusions in the darbepoetin alfa treatment group. A prespecified sensitivity analysis of the first occurrence of either a red blood cell transfusion or a hemoglobin concentration ≤ 8 g/dL yielded a statistically significant difference between treatment groups favoring darbepoetin alfa. Significantly more deaths occurred in the darbepoetin alfa vs. placebo group. An effect of imbalances in potentially important prognostic factors that were present at baseline could not be excluded.

5. Risk Assessment of Epoetin alfa and Other ESAs in Oncology Based on J&JPRD Analyses

As noted previously, Ortho Biotech Products, LP is responsible for the clinical development, marketing, and distribution of Procrit in the United States under license from Amgen. Amgen is the marketing authorization holder and manufacturer for Procrit.

Since the clinical development of epoetin alfa has been conducted by J&JPRD, they have provided an updated safety evaluation based on their clinical experience.

5.1 Safety of Epoetin alfa in the Treatment of Chemotherapy-induced Anemia

5.1.1 Meta-Analysis Methodology

To better define the study and patient-level characteristics associated with key safety events, a predefined pooled analysis was performed. This meta-analysis was based on all 12 completed, randomized, double-blind, placebo-controlled studies that were conducted with epoetin alfa for which J&JPRD has access to patient-level data. Eleven of these studies were conducted in subjects receiving chemotherapy, and 1 small study was conducted in subjects not receiving chemotherapy. Two of these 11 studies investigated hemoglobin targets above those recommended in the prescribing information. These studies have been previously described (J&JPRD FDA ODAC Briefing Information, 2004). No additional studies meeting all of the above criteria with patient-level data were available at the date of data cut-off for inclusion in the new analyses reported in this section.

Studies were classified as “anemia correction” or “anemia prevention” based on a combination of the following: 1) the entry hemoglobin concentration, 2) the hemoglobin range that treatment was intended to achieve, 3) the criterion for study drug dose escalation, 4) the definition of “hemoglobin response,” and 5) the hemoglobin at which study drug dosing was suspended and subsequently restarted. For “anemia correction,” the primary intent of the studies was to reduce transfusion utilization. Typically, study drug dosing was not escalated as long as there was a satisfactory hemoglobin response (usually a 1-g/dL hemoglobin increase from baseline value). Conversely, the intent of the “anemia prevention” studies was generally to keep subjects’ hemoglobin $\geq$ 12 g/dL using dose escalation (if the hemoglobin was below the target range), or by commencing

treatment when the hemoglobin concentration was above 12 g/dL and continuing treatment beyond the usual time frame of 12 to 16 weeks.

Because a single study (BEST) contributed a substantial proportion of the overall deaths, J&JPRD conducted a sensitivity analysis with this study removed from the analysis population. BEST was limited to female subjects with breast cancer, who were treated above hemoglobin levels recommended in the prescribing information, and used a weekly dosing regimen.

Table 9 identifies the studies that were used in the meta-analyses.

Table 9. Overview and Design of Completed, Double-Blind, Placebo-Controlled, Multicenter Clinical Studies Used in the Meta-Analysis

No.	Study	Tumor Type	Entry Hb (Hct)/ Upper Hb (Hct) Limit On Study	EPO SC Dose Regimen/ Dose Adjustment	No. of Subjects (DB Phase)[a] EPO	Placebo	Total
Completed, Double-Blind, Placebo-Controlled, Multicenter Clinical Studies of Anemia Correction							
1.	Non-CT (H87-032, 87-014, 87-015)	Mixed	Hb: ≤10.5 g/dL[b]/ Hct: 38%-40%	100 U/kg TIW for ≤8 wks/titrated to target	65	59	124
2.	Non-cisplatin CT (I88-037, 87-016, 87-017)	Mixed	Hb: ≤10.5 g/dL/ Hct: 38%-40%	150 U/kg TIW for ≤12 wks/titrated to target	81	76	157
3.	Cisplatin CT (I88-036, 87-018, 87-019)	Mixed	Hb: ≤10.5 g/dL/ Hct: 38%-40%	150 U/kg TIW for ≤12 wks/titrated to target	67	65	132
4.	J89-040	CLL	Hct: <32%/ Hct: 38%-40%	150 U/kg TIW for ≤12 wks/titrated to target	142	79	221
5.	CC2574-P-174	CLL	Hct: <32%/ Hct: 38%-40%	150 U/kg TIW for ≤12 wks/titrated to target	33	12	45
6.	EPO-INT-1[c,d]	Ovarian	Hb: <11.0 g/dL OR ↓ ≥1.5 g/dL (from BL <14.0 g/dL) OR ↓ ≥2.0 g/dL (from BL ≥14.0 g/dL)/ Hb: 12.5-14 g/dL + ↑<2 g/dL/mo	150 or 300 U/kg TIW for 1 month past last CT cycle/ EPO dose maintained based on reticulocyte count, Hb ↑, and Hb level; if dose held based on above, then restarted at 25% ↓ dose	165[d]	81	246
7.	EPO-INT-2[c]	MM	Hb: <11.0 g/dL/ Hb: 12-14 g/dL + ↑<2 g/dL/mo	150-300 U/kg TIW for 12 wks/ EPO dose ↑ if target Hb rise from BL not met; if dose held based on exceeding Hb criterion for dose hold, EPO restarted at 25% ↓ dose	69	76	145

Abbreviations: BL, baseline; DB, double-blind; Hb, hemoglobin; Hct, hematocrit; EPO, epoetin alfa; SC, subcutaneous; CT, chemotherapy; TIW, 3 times weekly; CLL, chronic lymphocytic leukemia; BL, baseline; MM, multiple myeloma; mo, month; QW, once weekly; wks, weeks; ↓, decreases; ↑, increases

[a] Actual number of subjcts enrolled.
[b] Under protocol 87-014.
[c] Data available on tumor response and disease progression.
[d] 80 subjects in 300-U/kg group and 85 subjects in 150-U/kg group.

Continued

Table 9. Overview and Design of Completed, Double-Blind, Placebo-Controlled, Multicenter Clinical Studies Used in the Meta-Analysis

No.	Study	Tumor Type	Entry Hb (Hct)/ Upper Hb (Hct) Limit On Study	EPO SC Dose Regimen/ Dose Adjustment	No. of Subjects (DB Phase)[a] EPO	Placebo	Total
Completed, Double-Blind, Placebo-Controlled, Multicenter Clinical Studies of Anemia Correction (Continued)							
8.	EPO-INT-3[c]	Mixed	Hb: <12.0 g/dL/ Hb: 14-16 g/dL (men), 12-14 g/dL (women) + ↑<2 g/dL/mo	150-300 U/kg TIW for 12 wks/ EPO dose ↑ if target Hb rise from BL not met; if dose held based on exceeding Hb criterion for dose hold, EPO restarted at 25% ↓ dose	136	65	201
9.	EPO-INT-10[c]	Mixed	Hb: ≤10.5 g/dL/ Hb: 12-15 g/dL + ↑<2 g/dL/mo	150-300 U/kg TIW for ≤6 cycles or 24 wks/ EPO dose ↑ based on reticulocyte count and target Hb ↑ not met; dose held based on exceeding Hb criterion for dose hold and restarted at 25% ↓ dose	251	124	375
10.	PR98-27-008[c]	Mixed	Hb: ≤11.5 g/dL (men), ≤10.5 g/dL (women)/ Hb: 13-15 g/dL	40,000 U QW for 16 wks/ EPO dose ↑ if Hb target rise not met or transfusion; dose held based on exceeding Hb criterion for dose hold and restarted at 25% ↓ dose	174	170	344
Completed, Double-Blind, Placebo-Controlled, Multicenter Clinical Studies Anemia Prevention							
11.	N93-004	SCLC	≤14.5 g/dL	150 U/kg SC TIW until 3 wks after completing their initial course of treatment	109	115	224
12.	EPO-INT-76 (BEST)[e]	Breast	No Hb limit specified for inclusion	40,000 U SC QW for 12 months	448	456	904

Abbreviations: BL, baseline; DB, double-blind; Hb, hemoglobin; Hct, hematocrit; EPO, epoetin alfa; SC, subcutaneous; CT, chemotherapy; TIW, 3 times weekly; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; mo, month; QW, once weekly; wks, weeks; ↓, decreases; ↑, increases

[a] Actual number of subjects enrolled.
[b] Under protocol 87-014.
[c] Data available on tumor response and disease progression.
[d] 80 subjects in 300-U/kg group and 85 subjects in 150-U/kg group.
[e] Study drug was discontinued in April 2002.

5.1.2 Survival

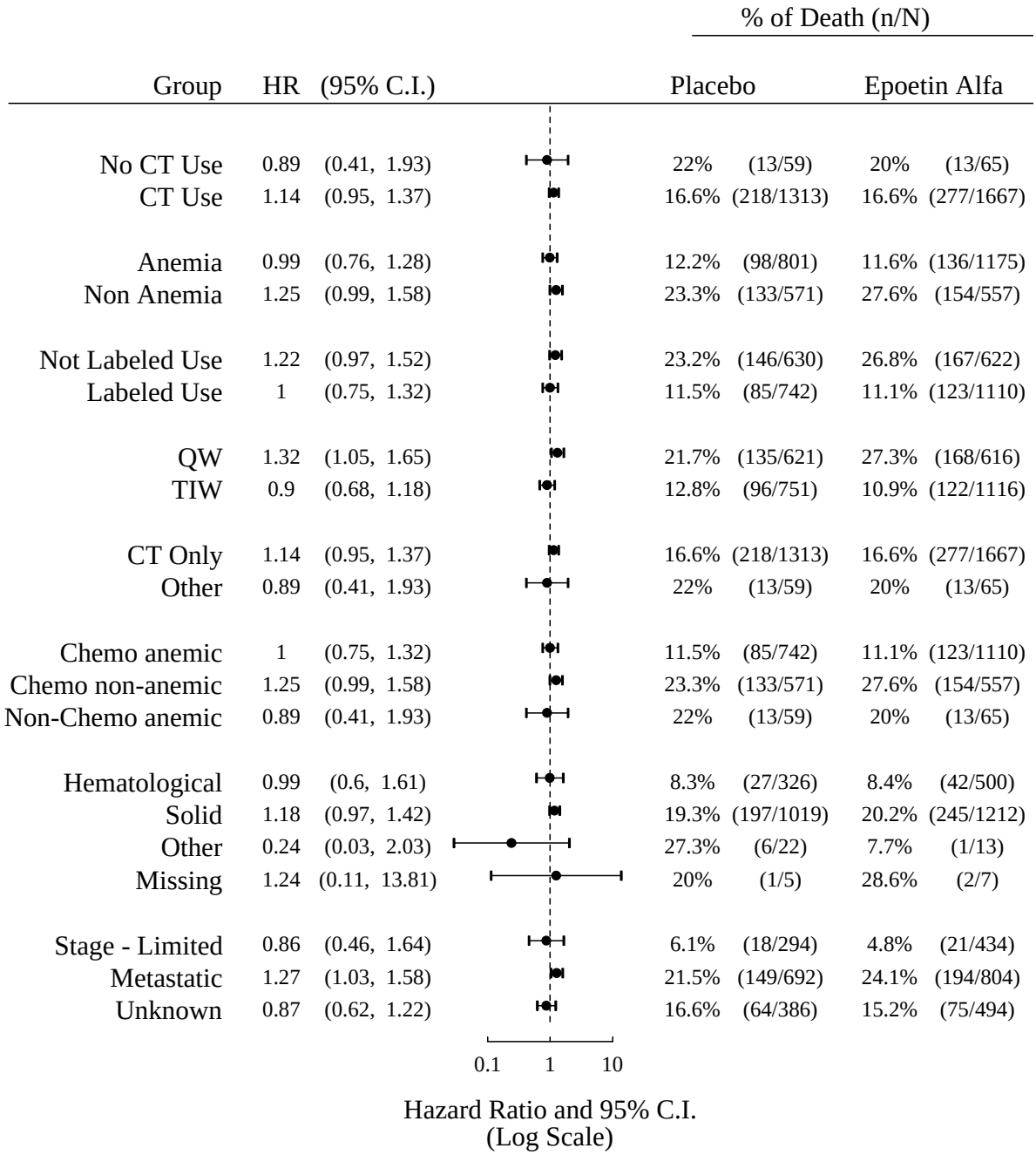
5.1.2.1 Study-Level, Patient-Level Subgroups

For the purposes of this ODAC review, J&JPRD has estimated hazard ratios for survival related to ESA use (on study plus 30 days) in subgroups of studies defined by relevant study characteristics (eg, treatment beyond the correction of anemia versus correction of anemia) and in subgroups of subjects defined by baseline patient-level characteristics. Patient-level characteristics were available for 3,104 subjects. These analyses are exploratory and hypothesis generating, and should be interpreted with caution. Results of these analyses are presented for study-level characteristics in Figure 21 and for patient-level characteristics in Figure 22.

Not all of the patient-level variables of interest were recorded in all of the early studies, so analyses of some covariates were performed in a subset of the studies.

The BEST study was the largest study included in the meta-analysis data set, contributing 904 of the total 3,104 subjects, and 246 of the 521 deaths. The BEST study addressed the weekly administration of epoetin alfa compared with placebo control, targeting a hemoglobin concentration above the correction of anemia in women with metastatic breast cancer. Many of the clinical factors that are linked to an apparently higher mortality associated with epoetin alfa therapy in the meta-analysis, including the diagnosis of breast cancer, a target hemoglobin of >12 g/dL, and the female sex, are all characteristics of the BEST study subject population.

Figure 21. Study-Level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase; Analysis Including BEST Study Data[a])



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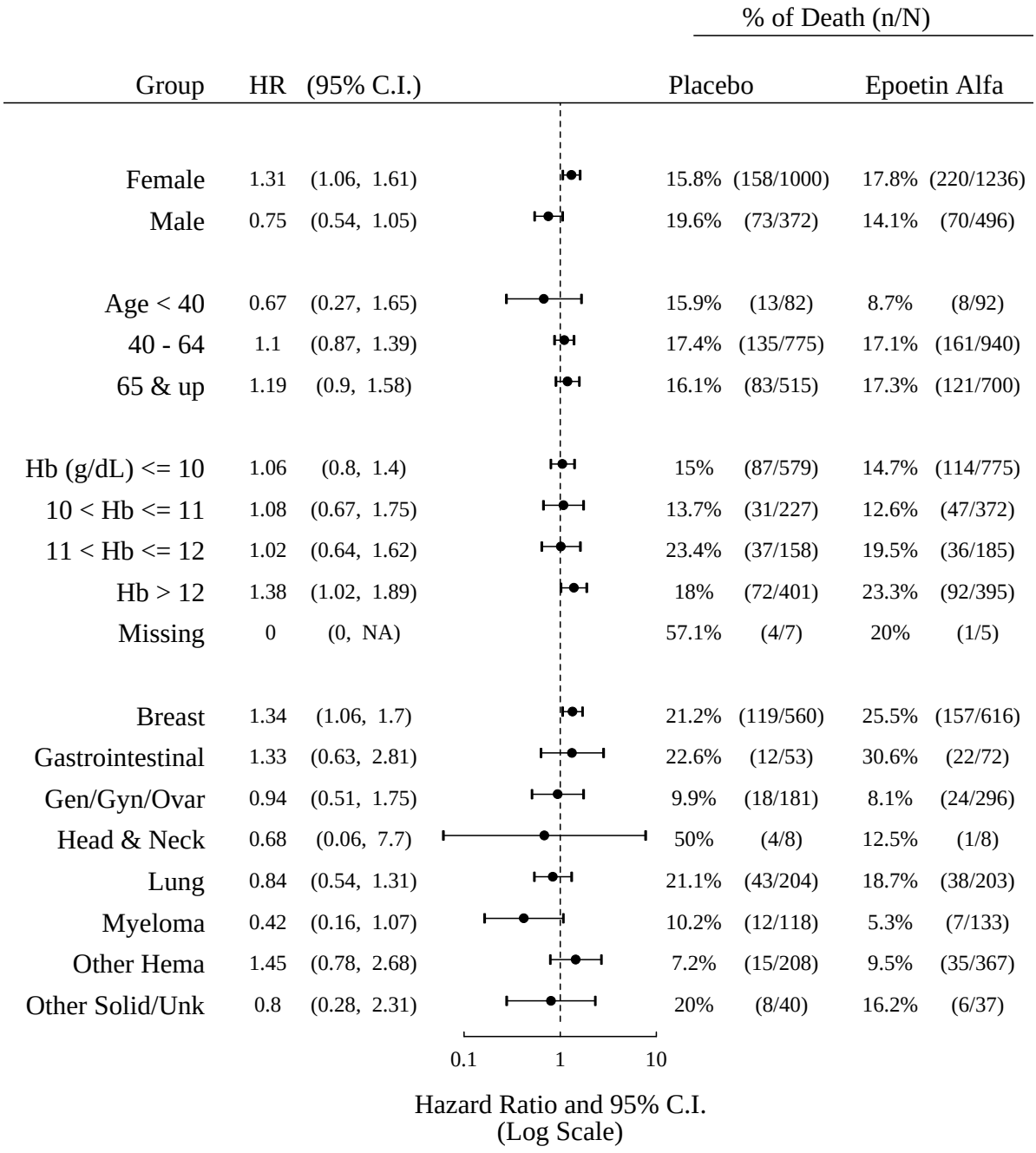
[a] For BEST, mortality was measured for up to 1 year from randomization + 2 weeks.
CT= chemotherapy; HR=hazard ratio; QW=once weekly, TIW=3 times weekly
Note for the following subgroups: "anemia" includes "chemo anemic" + "non-chemo anemic" studies; "Non-labeled use" includes "chemo non-anemic" + "non-chemo anemic"; "Labeled use" includes "chemo anemic" studies.

Figure 22. Patient-level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Including BEST Study Data [a])



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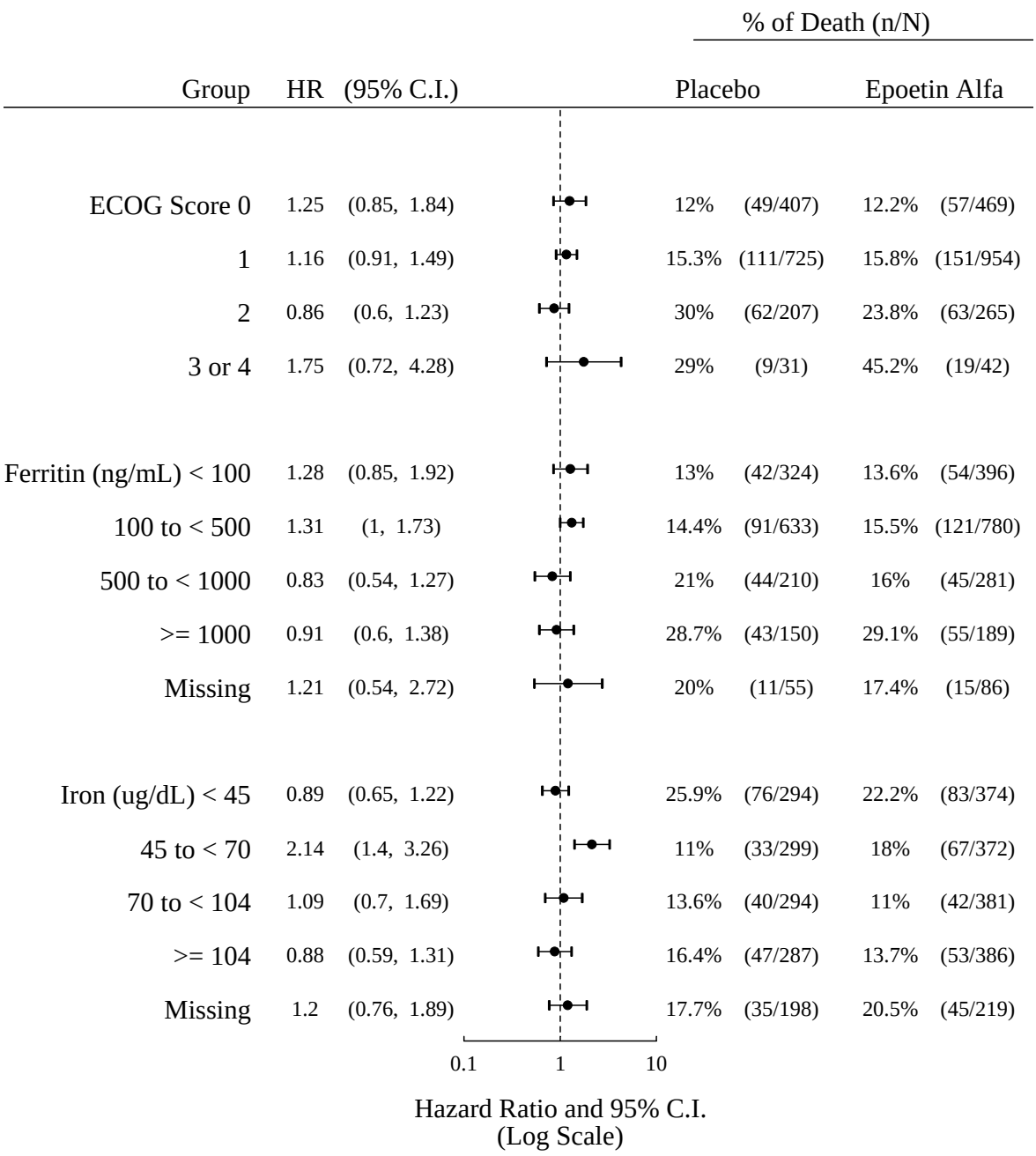
(Mon Apr 02 13:15:55 EDT 2007)

[a] For BEST, mortality was measured for up to 1 year from randomization + 2 weeks.
Gen=genital; gyn= gynecologic; Hgb=hemoglobin; hema=hematologic; ovar=ovarian; unk=unknown

Continued

Figure 22. Patient-level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Including BEST Study Data [a)]



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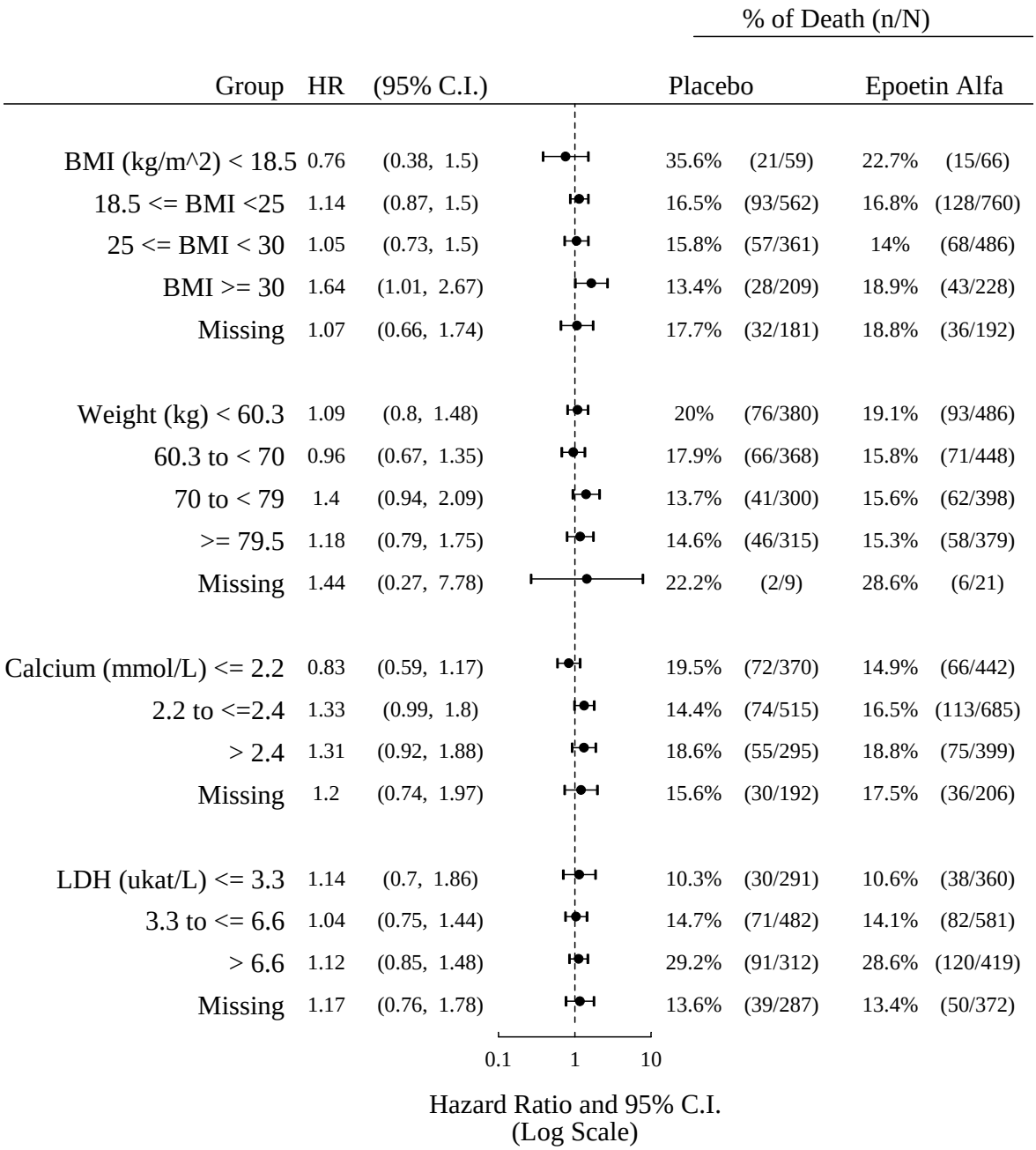
(Fri Mar 30 15:56:05 EDT 2007)

[a] For BEST, mortality was measured for up to 1 year from randomization + 2 weeks.
ECOG= Eastern Cooperative Oncology Group

Continued

Figure 22. Patient-level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Including BEST Study Data [a)]



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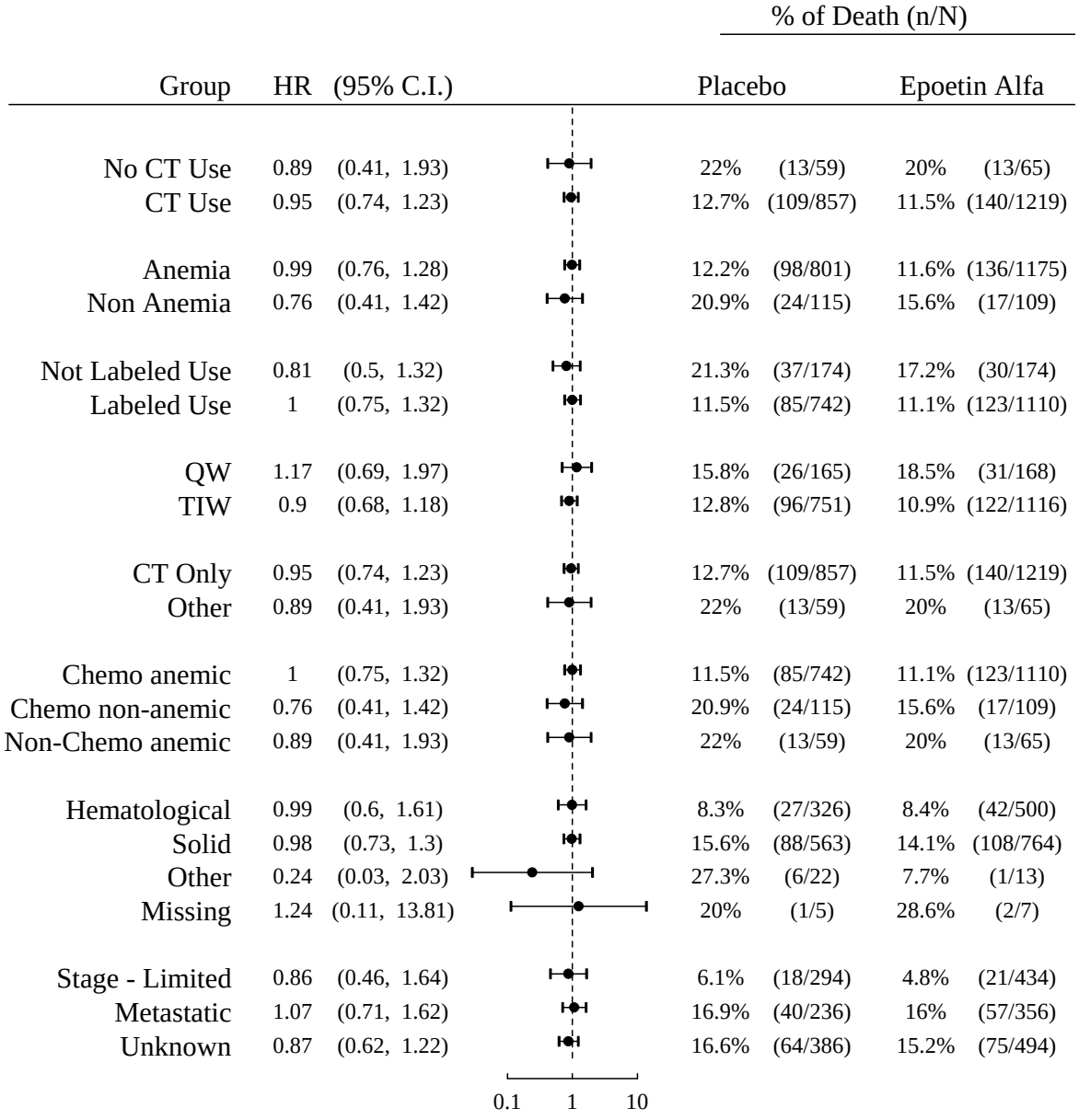
(Fri Mar 30 15:54:06 EDT 2007)

[a] For BEST, mortality was measured for up to 1 year from randomization + 2 weeks.
BMI=body mass index, LDH=lactate dehydrogenase

Figure 23. Study-Level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Not Including BEST Study Data)



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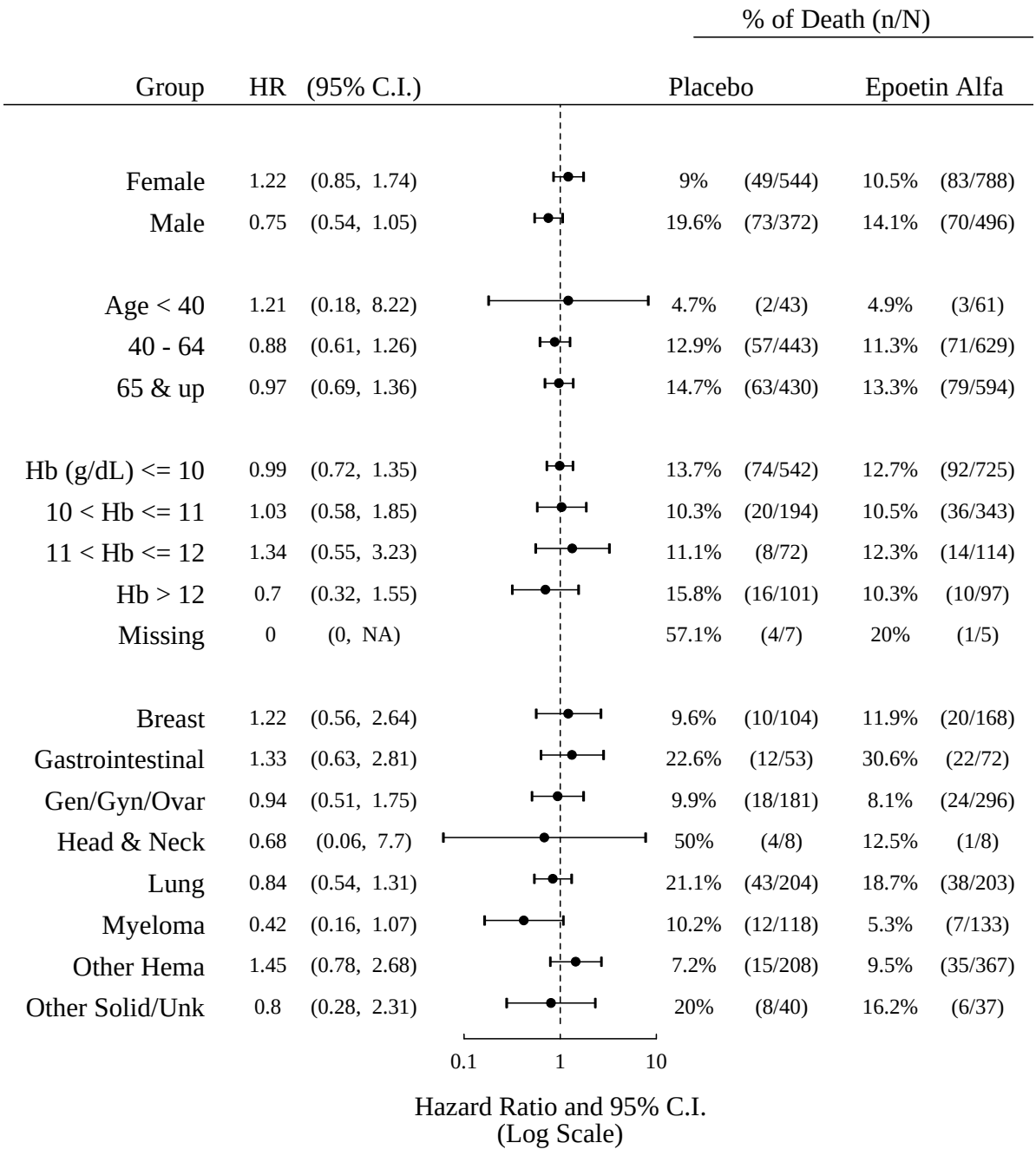
(Mon Apr 02 13:11:06 EDT 2007)

CT= chemotherapy; HR=hazard ratio; QW=once weekly, TIW=3 times weekly
Note for the following subgroups: “anemia” includes “chemo anemic” + “non-chemo anemic” studies; “Non-labeled use” includes “chemo non-anemic” + “non-chemo anemic”; “Labeled use” includes “chemo anemic” studies.

Figure 24. Patient-Level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Not Including BEST Study Data)



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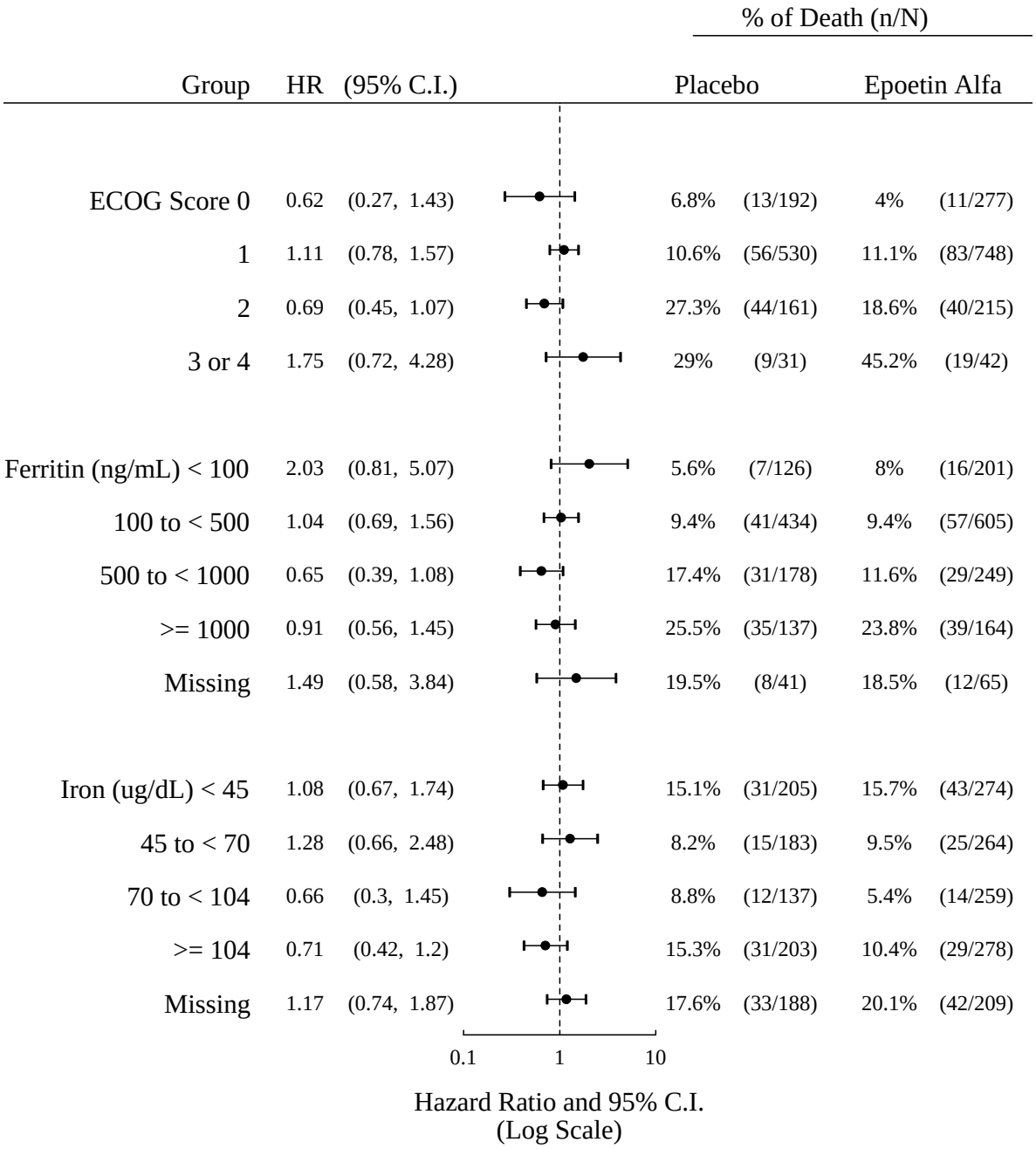
(Mon Apr 02 13:28:34 EDT 2007)

Gen=genital; gyn= gynecologic; hema=hematologic; Hgb=hemoglobin; HR=hazard ratio; ovar=ovarian; unk=unknown

Continued

Figure 24. Patient-Level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Not Including BEST Study Data)



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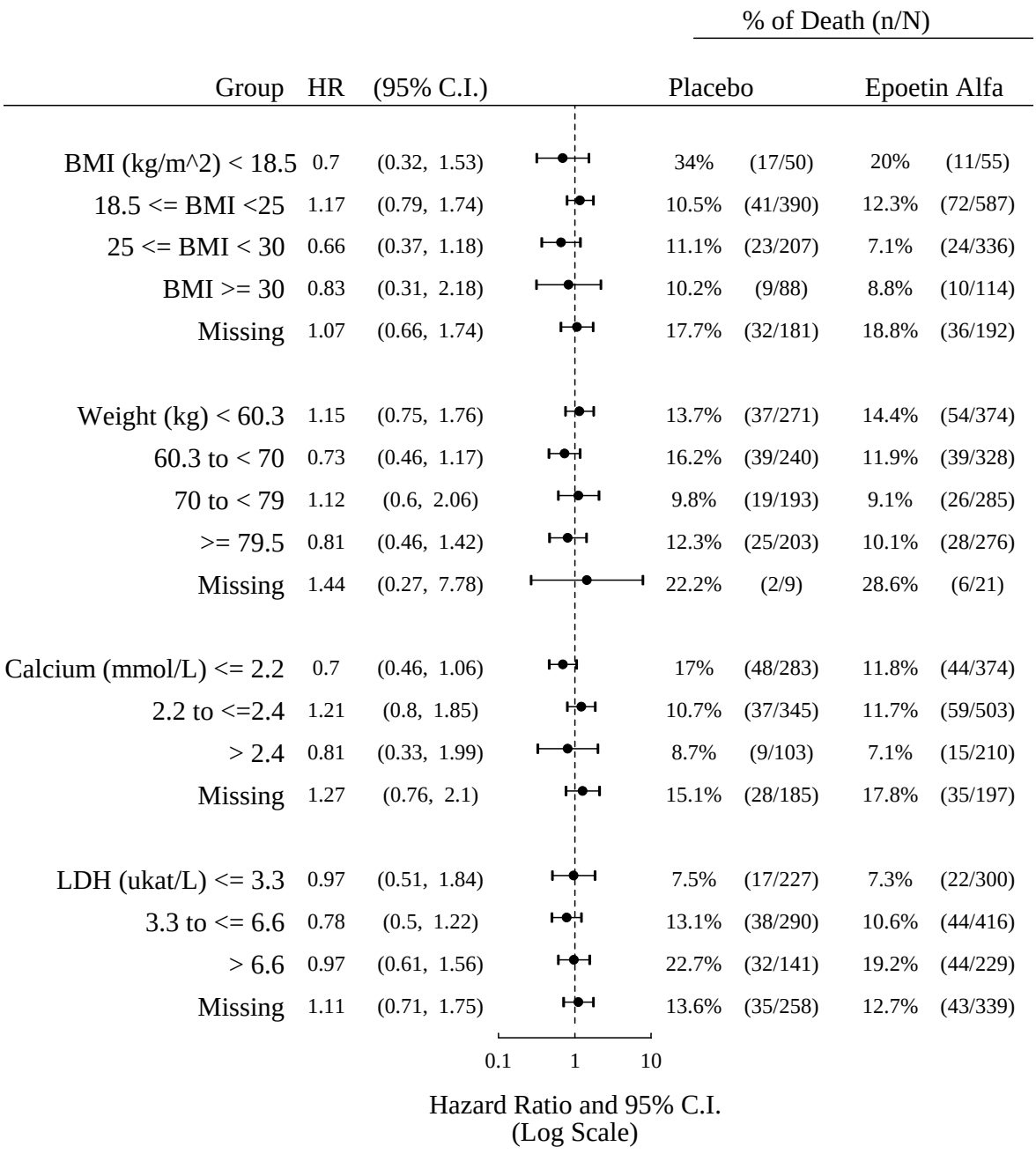
(Fri Mar 30 15:55:40 EDT 2007)

ECOG=Eastern Cooperative Oncology Group; HR=hazard ratio

Continued

Figure 24. Patient-Level Characteristics: Hazard Ratio and 95% CI (Mortality Within 30 Days of Double-Blind Phase, Analysis Not Including BEST Study Data)



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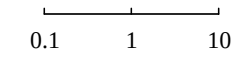
(Fri Mar 30 15:55:01 EDT 2007)

BMI=body mass index, HR=hazard ratio; LDH=lactate dehydrogenase

Figure 25. BEST Study Patient-Level Characteristics: Hazard Ratio and 95% CI (Mortality up to 1 year from Randomization + 2 weeks)

Group	HR	(95% C.I.)	% of Death (n/N) Placebo		Epoetin Alfa	
Age < 40	0.56	(0.2, 1.62)	28.2%	(11/39)	16.1%	(5/31)
40 - 64	1.29	(0.95, 1.75)	23.5%	(78/332)	28.9%	(90/311)
65 & up	1.9	(1.12, 3.24)	23.5%	(20/85)	39.6%	(42/106)
Hgb (g/dL) <= 10	1.45	(0.73, 2.87)	35.1%	(13/37)	44%	(22/50)
10 < Hb <= 11	1.18	(0.51, 2.72)	33.3%	(11/33)	37.9%	(11/29)
11 < Hb <= 12	0.91	(0.52, 1.58)	33.7%	(29/86)	31%	(22/71)
Hb > 12	1.58	(1.12, 2.21)	18.7%	(56/300)	27.5%	(82/298)
ECOG Score 0	1.51	(0.98, 2.34)	16.7%	(36/215)	24%	(46/192)
1	1.22	(0.85, 1.74)	28.2%	(55/195)	33%	(68/206)
2	1.33	(0.72, 2.47)	39.1%	(18/46)	46%	(23/50)
Ferritin (ng/mL) < 100	1.13	(0.71, 1.79)	17.7%	(35/198)	19.5%	(38/195)
100 to < 500	1.58	(1.09, 2.29)	25.1%	(50/199)	36.6%	(64/175)
500 to < 1000	1.37	(0.66, 2.86)	40.6%	(13/32)	50%	(16/32)
>= 1000	0.94	(0.4, 2.2)	61.5%	(8/13)	64%	(16/25)
Missing	0.64	(0.13, 3.2)	21.4%	(3/14)	14.3%	(3/21)
Iron (ug/dL) < 45	0.76	(0.5, 1.17)	50.6%	(45/89)	40%	(40/100)
45 to < 70	2.94	(1.69, 5.1)	15.5%	(18/116)	38.9%	(42/108)
70 to < 104	1.34	(0.79, 2.27)	17.8%	(28/157)	23%	(28/122)
>= 104	1.19	(0.63, 2.24)	19%	(16/84)	22.2%	(24/108)
Missing	1.69	(0.28, 10.12)	20%	(2/10)	30%	(3/10)
BMI (kg/m^2) < 18.5	1.01	(0.25, 4.03)	44.4%	(4/9)	36.4%	(4/11)
18.5 <= BMI <25	1.12	(0.76, 1.63)	30.2%	(52/172)	32.4%	(56/173)
25 <= BMI < 30	1.37	(0.88, 2.15)	22.1%	(34/154)	29.3%	(44/150)
BMI >= 30	2.05	(1.16, 3.6)	15.7%	(19/121)	28.9%	(33/114)
Weight (kg) < 60.3	1.03	(0.66, 1.6)	35.8%	(39/109)	34.8%	(39/112)
60.3 to < 70	1.31	(0.78, 2.18)	21.1%	(27/128)	26.7%	(32/120)
70 to < 79	1.65	(0.97, 2.81)	20.6%	(22/107)	31.9%	(36/113)
>= 79.5	1.68	(0.96, 2.94)	18.8%	(21/112)	29.1%	(30/103)
Calcium (mmol/L) <= 2.2	1.18	(0.66, 2.11)	27.6%	(24/87)	32.4%	(22/68)
2.2 to <=2.4	1.46	(0.96, 2.22)	21.8%	(37/170)	29.7%	(54/182)
> 2.4	1.43	(0.97, 2.09)	24%	(46/192)	31.7%	(60/189)
Missing	0.42	(0.04, 4.61)	28.6%	(2/7)	11.1%	(1/9)
LDH (ukat/L) <= 3.3	1.42	(0.68, 2.95)	20.3%	(13/64)	26.7%	(16/60)
3.3 to <= 6.6	1.41	(0.88, 2.24)	17.2%	(33/192)	23%	(38/165)
> 6.6	1.21	(0.86, 1.7)	34.5%	(59/171)	40%	(76/190)
Missing	1.62	(0.47, 5.54)	13.8%	(4/29)	21.2%	(7/33)



Hazard Ratio and 95% C.I.
(Log Scale)

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BMI=body mass index; ECOG= Eastern Cooperative Oncology Group; HR=hazard ratio; LDH=lactate dehydrogenase

The subgroup effects associated with these characteristics of the BEST study cannot be distinguished from each other because those characteristics are nearly completely confounded with each other because of confounding. In other words, given the adverse mortality effect observed in the BEST study, the major component of the meta-analysis, it is not surprising that factors associated with mortality in the BEST study would also be associated with mortality in the overall meta-analysis. Consequently, the meta-analysis has limited ability to distinguish among the factors identified above in terms of which may be causally associated with mortality. The apparent subgroup effects in the above meta-analyses were no longer evident in the sensitivity analyses with BEST study data removed (Figures 23 and 24).

In the BEST study, baseline BMI ≥30 or hemoglobin concentration >12 g/dL were linked to increased hazard ratios for mortality associated with epoetin alfa use. However, these characteristics were linked to numerically lower overall 1-year mortality (Figure 25) in both groups. Expressed another way, although higher BMI and hemoglobin appeared to be associated with numerically lower mortality when analyzed within treatment groups, these factors also appeared to identify subgroups with an increased hazard ratio for mortality associated with epoetin alfa treatment. Interestingly, the subgroup with high BMI also had a higher hazard ratio for risk of TVEs associated with epoetin alfa therapy.

ESA Response and Survival

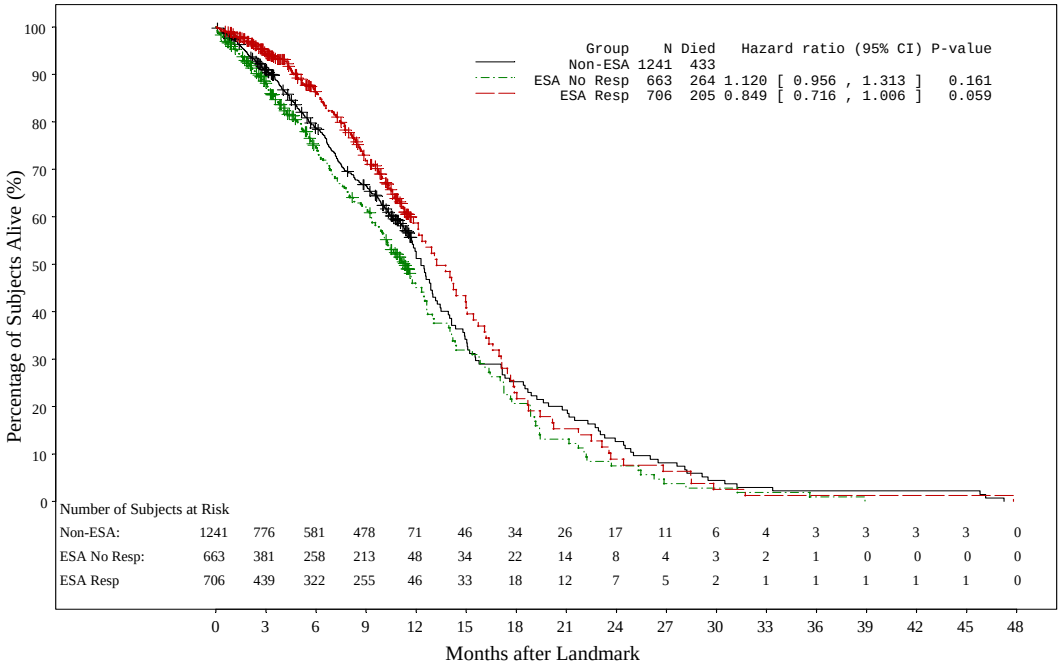
ESA dose escalation is highly correlated with non-response to treatment, ie, absence of a hemoglobin increase of at least 1 g/dL over baseline. In these studies, escalated doses were sometimes administered to subjects who failed to respond within the first few weeks of treatment. Thus, examination of dose-toxicity relationships is potentially confounded by subject characteristics that determine response to ESAs.

To test the hypothesis that safety outcomes in randomized studies of epoetin alfa might differ between subjects whose hemoglobin increased (“hemoglobin responders”) compared with those whose hemoglobin failed to increase (“hemoglobin non-responders”), an exploratory analysis was conducted comparing the survival and TVE experience of these subject subsets with placebo. Specifically, a “landmark analysis” was used, which defines a hemoglobin responder at a pre-specified point in time (the landmark), and then examines survival subsequent to that point in time.

To address potential bias in the choice of the time point defining the landmark, pre-landmark survival was compared between the treated and control subjects, and the analyses were also performed using 2 choices of landmark, 4 weeks and 8 weeks. The 4-week analysis defines hemoglobin non-response strictly in terms of increases in hemoglobin concentrations at 4 weeks after the start of treatment. This definition avoids confounding by dose escalation, which typically occurs at 4 to 6 weeks, for subjects who have not had a sufficient rise in hemoglobin at that point. The 8-week definition of non-response will, for most non-responding subjects, incorporate dose-escalation as an inherent (implicit) component of the definition.

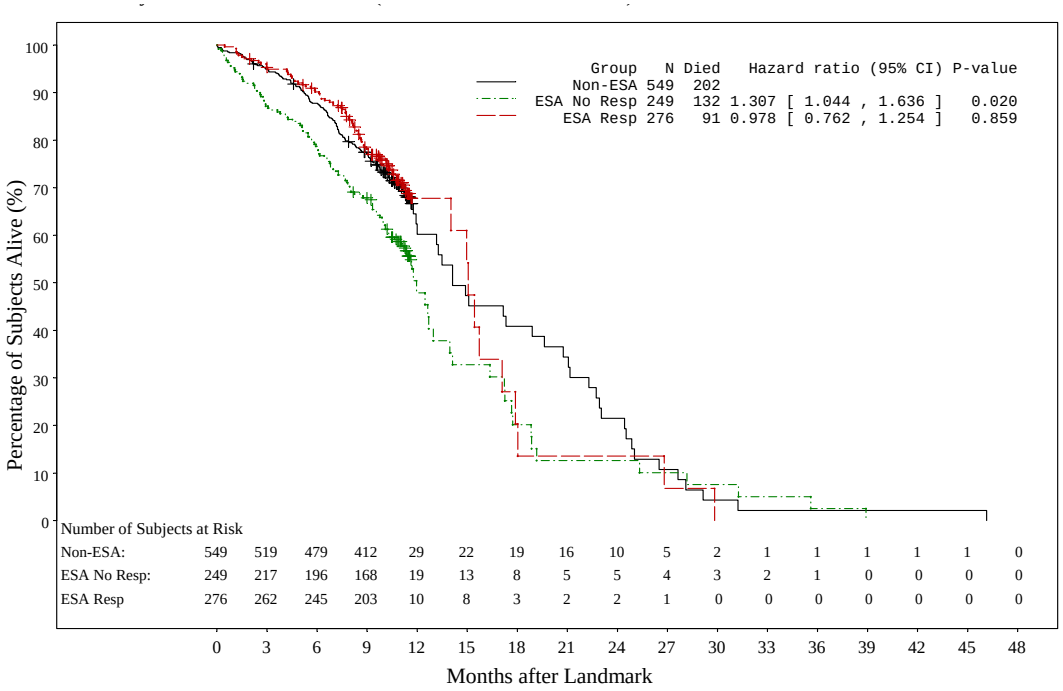
Survival was estimated using the Kaplan-Meier method and comparisons were made between the responders and non-responders versus the placebo group (Figure 26 to Figure 28). Cox's proportional hazards model was used to adjust for the following baseline covariates: baseline hemoglobin prior to treatment start, baseline performance status, and advanced disease at baseline (yes vs. no). All analyses were stratified by study to account for any differences in the study populations and study conduct.
A sensitivity analysis was also performed in which the two anemia prevention studies (BEST, N93-004) were removed (Figure 29).

Figure 26. Survival and ESA Response: 4-Week Landmark Analysis (All 12 Double-Blind, Placebo-Controlled Studies: Landmark at 4 Weeks on Treatment)



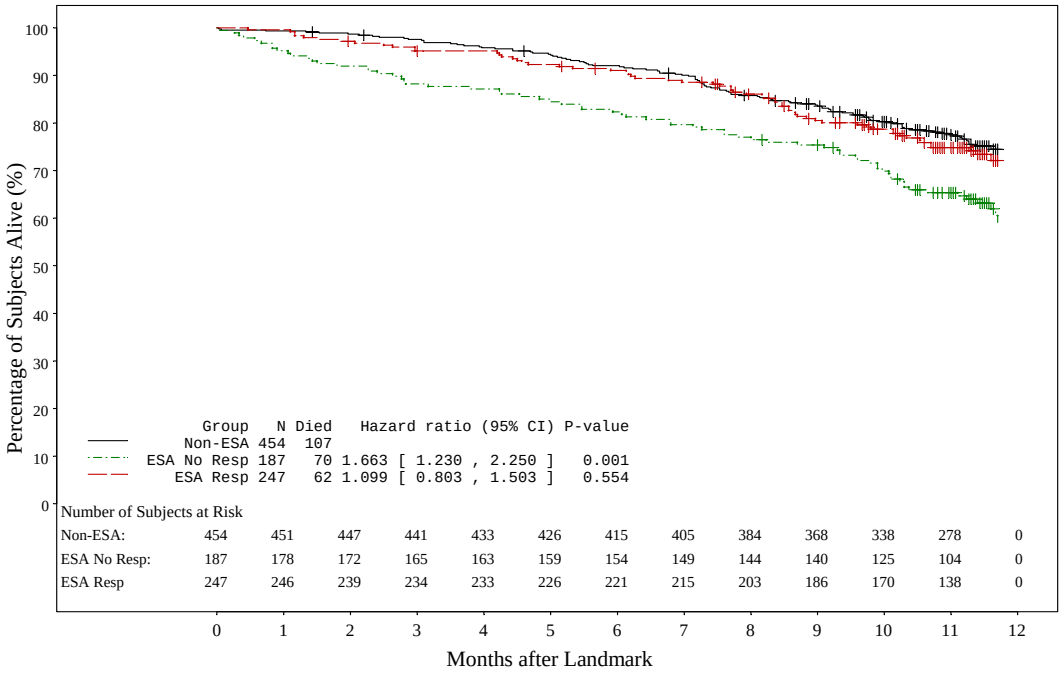
Note: 1) Hazards ratios p values are based on a proportional hazards model that is stratified by study and included Eastern Cooperative Oncology Group score, pretreatment hemoglobin, tumor type, and advanced disease. 2) Response is defined as ≥1 g/dL increase in hemoglobin within 4 weeks, independent of transfusion.

Figure 27. Survival and ESA Response: 4-Week Landmark Analysis (Anemia prevention [BEST and EPO-N93-004]; Landmark at 4 Weeks on Treatment)



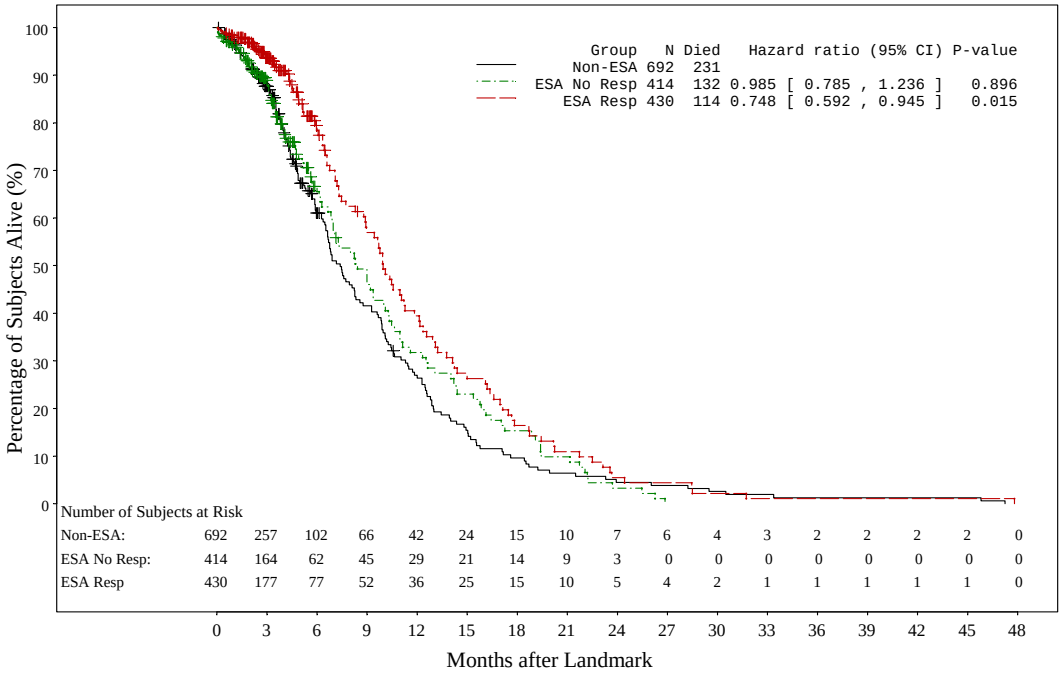
Note: 1) Hazards ratios p values are based on a proportional hazards model that is stratified by study and included Eastern Cooperative Oncology Group score, pretreatment hemoglobin, and advanced disease. 2) Response is defined as ≥1 g/dL increase in hemoglobin within 4 weeks, independent of transfusion.

Figure 28. Survival and ESA Response: 4-Week Landmark Analysis (BEST: Landmark at 4 Weeks on Treatment)



Note: 1) Hazards ratios p values are based on a proportional hazards model that adjusted for Eastern Cooperative Oncology Group score and pretreatment hemoglobin 2) Response is defined as ≥1 g/dL increase in hemoglobin within 4 weeks, independent of transfusion.

Figure 29. Survival and ESA Response: 4-Week Landmark Analysis (10 Double-Blind, Anemia-Correction Studies: Landmark at 4 Weeks on Treatment)



Note: 1) Hazards ratios p values are based on a proportional hazards model that is stratified by study and included Eastern Cooperative Oncology Group score, pretreatment hemoglobin, and advanced disease. 2) Response is defined as ≥1 g/dL increase in hemoglobin within 4 weeks, independent of transfusion.

These exploratory findings (Figure 26 to Figure 29) suggest the possibility that patients identified as non-responders to ESAs are at increased risk of death. While responders appeared to have better outcomes, is should be noted that in a large study aimed at preventing anemia (BEST), subjects treated to hemoglobin targets above those recommended in the prescribing information appeared to have a higher mortality. Although these analyses were adjusted for several key baseline covariates, it is unclear whether these effects result from treatment, or whether patients who fail to respond to epoetin alfa are inherently at increased risk of death (eg, due to unmeasured characteristics of the underlying malignancy), regardless of their treatment status. Overall these exploratory analysis suggest that patients who achieve higher on study hemoglobin levels have improved survival outcomes. These results do not support the achieved hemoglobin level as the predictor of risk of worsened survival outcomes.

5.1.2.2 Conclusion: Survival

When used according to labeled guidance for correction of anemia in the setting of CIA, there is no evidence that epoetin alfa has an adverse effect on survival. However, in the

BEST study of anemia prevention, epoetin alfa was associated with an increased risk for death and serious CV/TE events when administered to target a hemoglobin concentration > 12 g/dL.

5.1.3 Thrombotic Vascular Events, Study and Patient-Level Subgroups

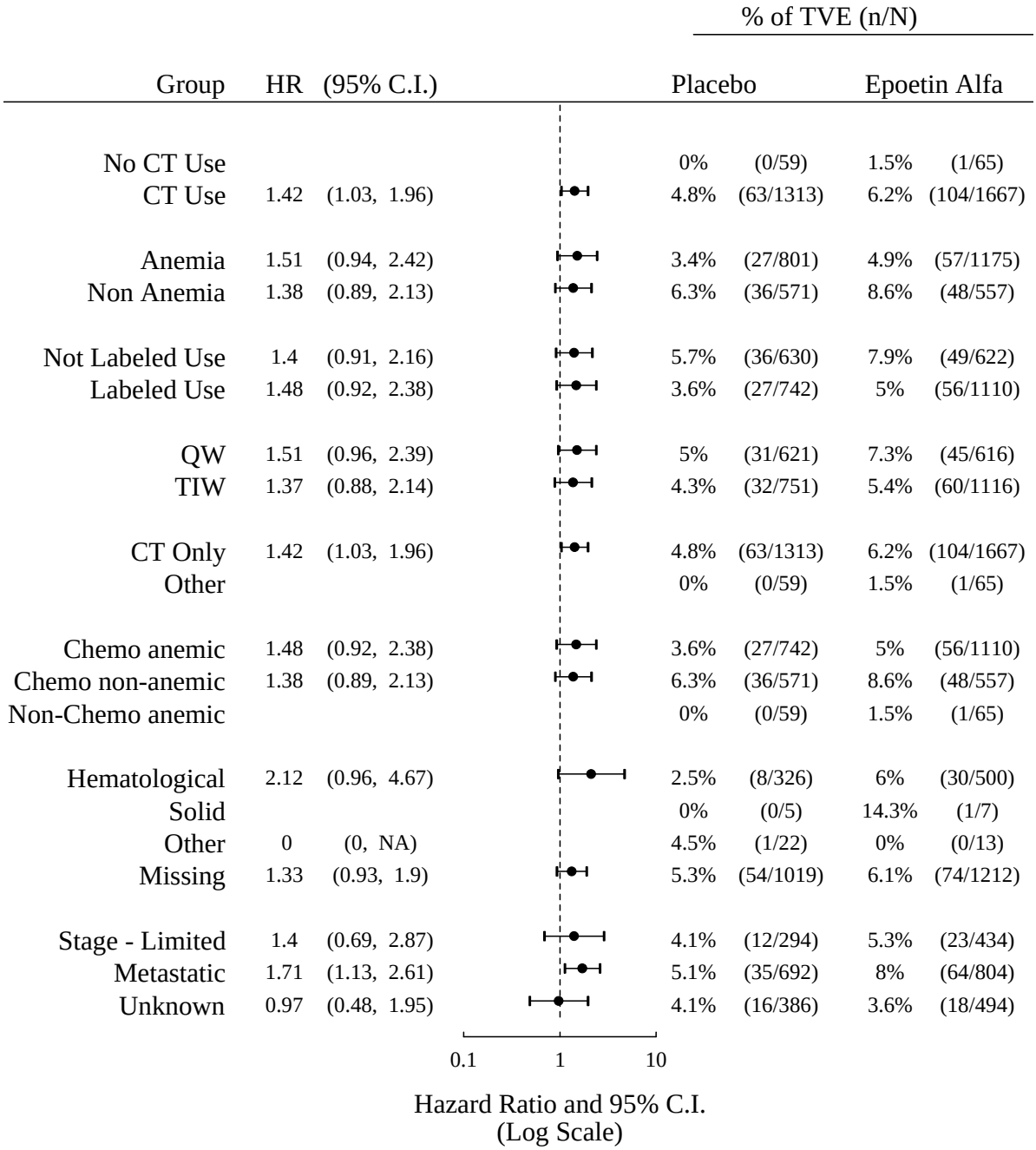
J&JPRD has estimated hazard ratios for clinically relevant TVEs in subgroups of studies defined by relevant study characteristics (eg, treatment for prevention of anemia versus correction of anemia) and by baseline patient-level characteristics.

These analyses are exploratory and hypothesis generating, and should be interpreted with caution. The results of these analyses for all 12 studies combined are presented for study-level and patient-level characteristics in Figure 30 and Figure 31, respectively.

Not all of the patient-level variables of interest were recorded in all of the early studies, so analyses of some covariates were performed in a subset of the studies.

The overall hazard ratio for TVE occurrence for epoetin alfa compared with placebo was 1.44 (95% CI: 1.05 to 1.98), consistent with previous experience in this population and already reflected in J&JPRD's product label. These analyses are difficult to interpret because of the small number of TVEs in each subcategory. No strong association of baseline Hgb and risk of TVE was observed. The results of the BEST study are again evident as dominant factors driving the association of breast cancer and female gender with adverse TVE outcomes. In general, the results by sub-group are broadly similar to the underlying overall risk (HR 1.44) for epoetin alfa treated patients and are concordant with the labeled rates of such events.

Figure 30. Study-Level Characteristics: Hazard Ratio and 95% CI (Clinically Relevant Thrombotic Vascular Events; Analysis Including BEST Study Data)



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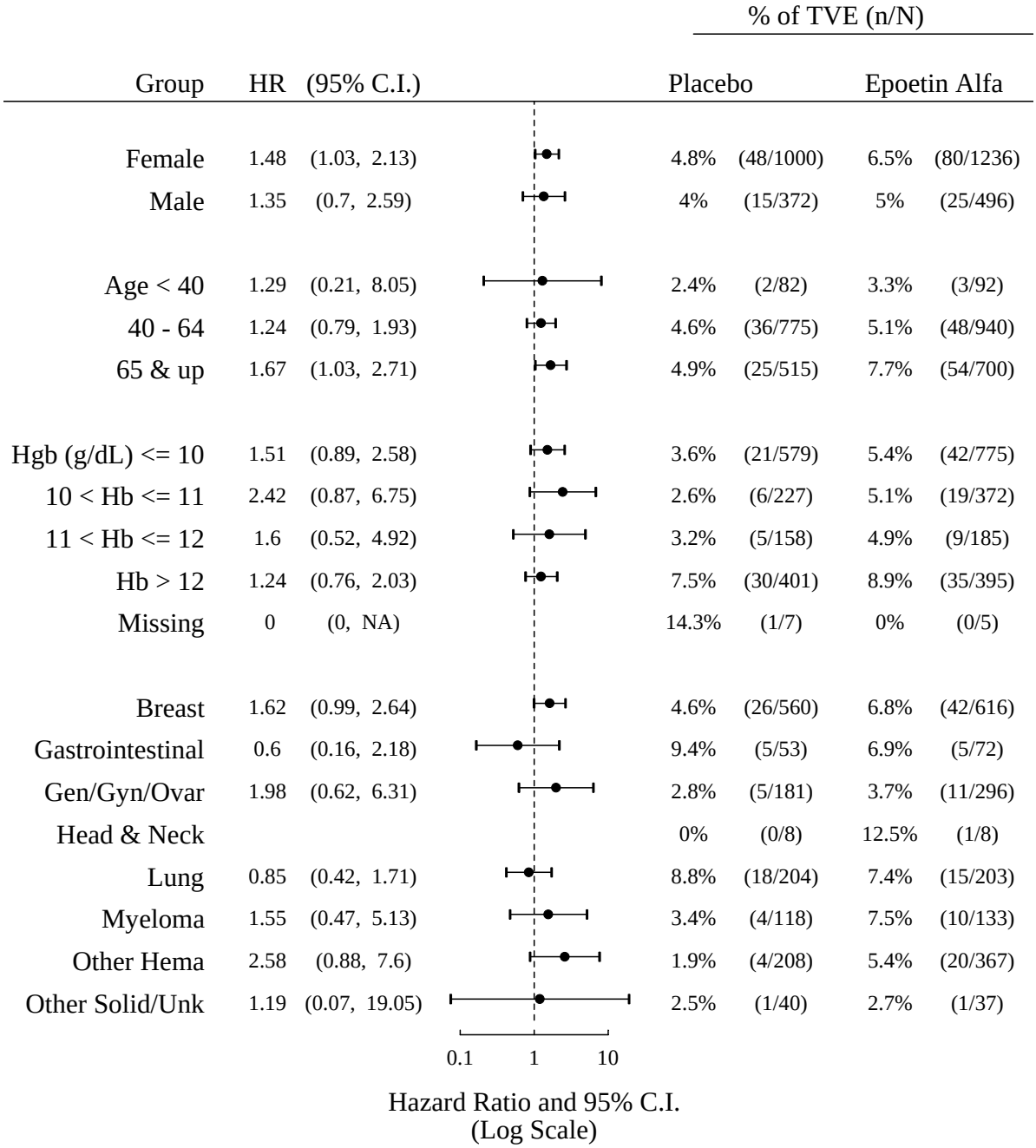
CT= chemotherapy; HR=hazard ratio; QW=once weekly, TIW=3 times weekly
Note for the following subgroups: "anemia" includes "chemo anemic" + "non-chemo anemic" studies; "Non-labeled use" includes "chemo non-anemic" + "non-chemo anemic"; "Labeled use" includes "chemo anemic" studies.

Figure 31. Patient-Level Characteristics: Hazard Ratio and 95% CI (Clinically Relevant Thrombotic Vascular Events; Analysis Including BEST Study Data)



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Gen=genital; gyn= gynecologic; Hgb=hemoglobin; HR=hazard ratio; ovar=ovarian; unk=unknown

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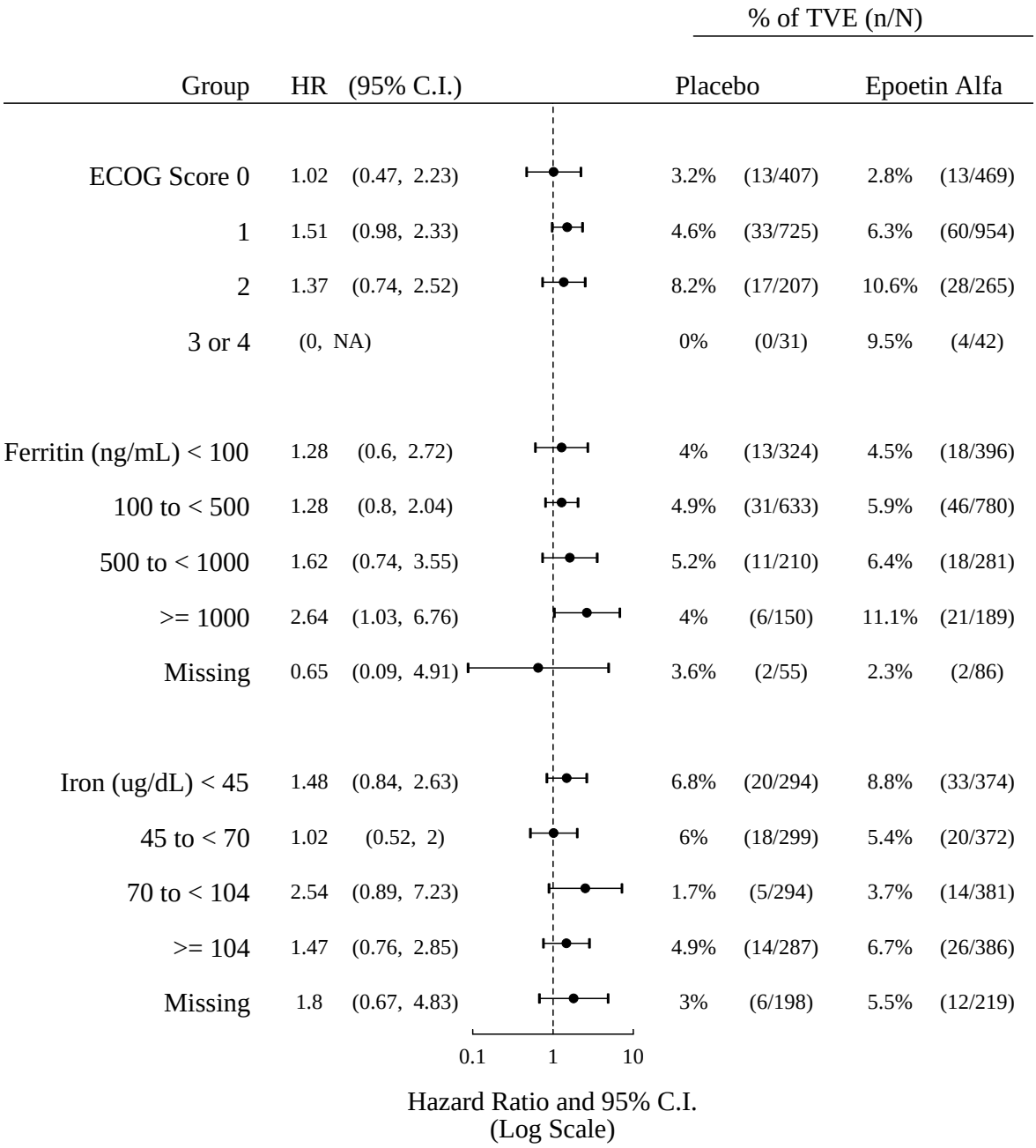
Figure 31. Patient-Level Characteristics: Hazard Ratio and 95% CI (Clinically Relevant Thrombotic Vascular Events; Analysis Including BEST Study Data)



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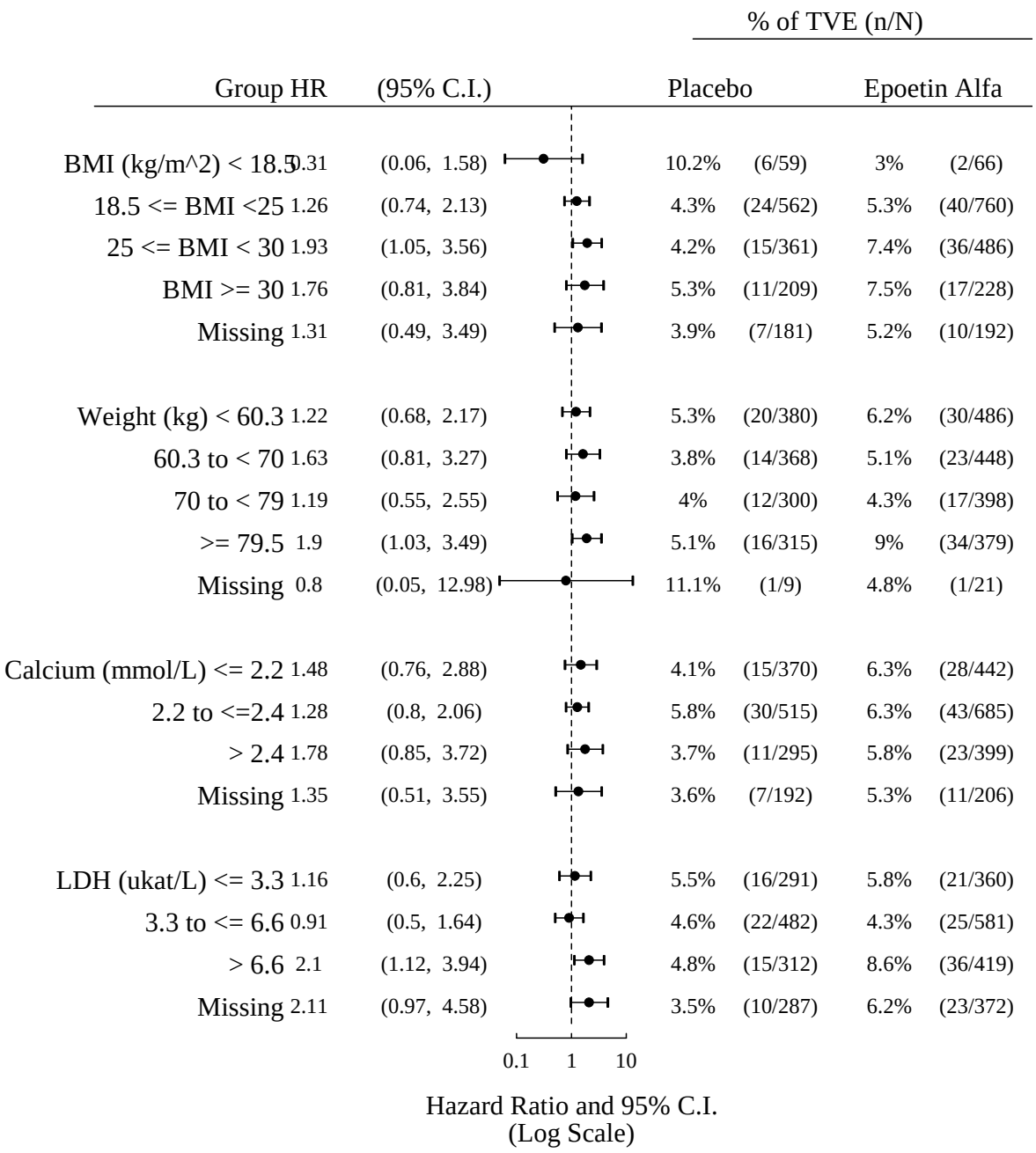
(Fri Mar 30 15:59:59 EDT 2007)

ECOG= Eastern Cooperative Oncology Group; HR=hazard ratio

Continued

Figure 31. Patient-Level Characteristics: Hazard Ratio and 95% CI (Clinically Relevant Thrombotic Vascular Events; Analysis Including BEST Study Data)



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BMI=body mass index; HR=hazard ratio; LDH=lactate dehydrogenase

ESA Response and TVE

The methods used to perform the landmark analysis of clinically relevant TVEs are similar to those described in the survival analysis section (see Section 5.1.2). J&JPRD's current definitions of clinically relevant TVE were used in the analysis. Results are presented in Table 10. As with the subgroup analyses above, these analyses are hard to interpret because of the small number of events and wide confidence intervals. The results are broadly consistent with the known increase in risk of TVEs in subjects treated with ESAs. However, no consistent pattern of increased risk among hemoglobin responders or non-responders is evident.

Table 10. Association Between Thrombotic Vascular Events and ESA Response[a]: Landmark Analysis (12 Double-Blind Placebo-Controlled Studies: All Treated Patients, Landmark at 4 Weeks on Treatment)

Study/Contrast	Hazards Ratio (b)	95% Confidence Interval (b)	P value (b)
All 12 DB Placebo-Controlled Studies			
ESA Non-Responders vs. Placebo	1.2072	(0.7452, 1.9555)	0.4442
ESA Responders vs. Placebo	1.4944	(0.9488, 2.3538)	0.0831
Beyond Anemia Correction			
ESA Non-Responders vs. Placebo	1.4223	(0.7915, 2.5556)	0.2388
ESA Responders vs. Placebo	1.1155	(0.6046, 2.0584)	0.7265
EPO-INT-76			
ESA Non-Responders vs. Placebo	1.5384	(0.7955, 2.9748)	0.2005
ESA Responders vs. Placebo	0.8364	(0.4096, 1.7077)	0.6237
10 DB Anemia-Correction Studies			
ESA Non-Responders vs. Placebo	0.9473	(0.4082, 2.1983)	0.8997
ESA Responders vs. Placebo	2.1756	(1.0673, 4.4347)	0.0324

Note:
a) ESA response is defined as >=1 g/dL increase in hemoglobin over pre-treatment level, independent of transfusion.
b) Analyses were based on Cox's proporional hazards model, adjusting for ECOG score, cancer type, advanced disease, and pre-treatment hemoglobin, stratified by study.

5.1.3.1 Conclusion: TVEs

There is an increased risk for TVEs among subjects with cancer who receive ESAs for the treatment of CIA. This is reflected in product labeling worldwide. J&JPRD continues to collect TVE information utilizing a standard data collection instrument to facilitate

future analyses of data regarding this safety risk. Treatment to target a hemoglobin of greater than 12 g/dL leads to greater risk and is strongly warned against in all of J&JPRD's product labeling.

5.1.4 Tumor Progression

5.1.4.1 Clinical Studies

In the BEST study, mortality at 4 months (8.7% versus 3.4%) was significantly higher in the epoetin alfa group versus the placebo group. The most common investigator-attributed cause of death within the first 4 months was disease progression, with 28 of 41 deaths in the epoetin alfa group and 13 of 16 deaths in the placebo group attributed to that cause. It is important to note, however, that the investigator assessment of time to tumor progression was not different between the two groups.

5.1.4.2 Exploratory Analyses

J&JPRD has not prepared any additional analyses to evaluate tumor progression as a safety endpoint because of the limited data available from completed studies.

5.1.4.3 Conclusions: Tumor Progression

Tumor progression as a basis for excess mortality observed in some clinical studies remains an unresolved issue. Although theoretically possible, it has not been consistently supported by preclinical data or in clinical studies within the indication that have been formally designed to assess this outcome.

Additionally, clinical studies within the labeled indication of CIA, in which tumor response has been measured, have not reported an adverse effect of epoetin alfa on either tumor progression or response to chemotherapy. J&JPRD is committed to evaluating the impact of ESAs on tumor progression in appropriately designed clinical studies.

5.1.5 Overall Safety Conclusion – Chemotherapy-induced Anemia

When used according to labeled guidance for correction of anemia in the setting of CIA, there is no evidence that epoetin alfa has an adverse effect on survival. However, in the BEST study of anemia prevention, epoetin alfa was associated with an increased risk for death and serious CV/TE events when administered to target a hemoglobin concentration > 12 g/dL. Data are more limited regarding tumor response and disease progression in the evaluated studies because they were not designed to formally assess this outcome. Nevertheless, the available studies in CIA in which time to tumor

progression has been evaluated have not revealed an adverse effect of epoetin alfa on either tumor progression or response to chemotherapy.

Labeling for all ESAs available in the United States accurately describes the association of TVEs with use of these products and provides an adequate measure of caution to prescribers in the form of a boxed warning. Experience in clinical studies of epoetin alfa in treatment of anemia in patients with cancer receiving chemotherapy, and information obtained from post-marketing surveillance, is consistent with this product labeling.

5.2 ESAs as an Adjunct to Radiotherapy

5.2.1 Theoretical Basis / Rationale

In contrast to CIA, where ESA use is supported by well-controlled clinical study evidence, the use of ESAs as an adjunct to therapeutic radiotherapy represents an entirely different (and currently still investigational) treatment paradigm.

It has long been recognized that the response of tumors to radiotherapy in patients with anemia is less satisfactory than in those with normal hemoglobin concentrations (McCormack et al, 1990).

Evans and Bergsjo (1965) found that for Stage II and Stage III disease in patients with carcinoma of the cervix, there was significantly lower survival in those patients who were anemic at the time of treatment compared with non-anemic patients. A report by Bush et al (1978) showed that in a study of 2,803 patients treated for carcinoma of the cervix with radiotherapy, those patients with hemoglobin concentrations below 12.0 g/dL had a poorer prognosis than those with higher hemoglobin concentrations. Dische (1991) reviewed the relationship between hemoglobin concentration and local tumor control in 25 studies. In the 23 studies involving patients with cancers of the uterine cervix, head and neck, lung or bladder, he found that the hemoglobin concentration was significantly predictive ($p < 0.05$) of local tumor response. In the other 2 studies, in patients with either brain (gliomas) or prostate cancer, there was no relationship.

The relationship of anemia to adverse outcome in patients receiving radiotherapy is proposed to be related to intratumoral hypoxia, with decreased local oxygen, a radiosensitizer (Molls et al, 1998; Strauss et al, 1999; Brizel et al, 1999). Hypoxia may lead to resistance to, or decreased efficacy of, chemotherapy, radiation therapy, or both.

5.2.2 Clinical Studies: Head and Neck Cancer

The ENHANCE and DAHANCA 10 studies in head and neck cancer, where ESAs were used to increase hemoglobin as an adjunct to therapeutic radiotherapy, have shown an adverse effect on outcome as previously described (see Section 2.4 and Section 2.5). J&JPRD has also provided updated information on 2 additional studies in patients with squamous cell carcinoma of the head and neck, one of which was terminated prematurely due to a safety signal.

5.2.2.1 Study RTOG 99-03

This was an open-label, cooperative group-sponsored study in which subjects with non-operative squamous cell carcinoma of the head and neck were randomly assigned 1:1 to Procrit 40,000 IU by SC administration once weekly plus radiotherapy or radiotherapy alone (Machtay, J&JPRD data on file). Planned enrollment was 372 subjects. The study was intended to determine whether treatment with epoetin alfa to maintain relatively high hemoglobin levels (up to 14 g/dL in women, up to 16 g/dL in men) would enhance the effectiveness of radiation therapy and improve local regional control. Analyses of 1-year actuarial local-regional control (HR: 1.18; 95% CI: 0.67 to 2.09), 1-year actuarial local-regional progression free survival (HR: 1.10; 95% CI: 0.65 to 1.89), and 1-year actuarial overall survival (HR: 1.57; 95% CI: 0.76 to 3.27) showed non-significant imbalances favoring the placebo group. These findings, together with the findings of the ENHANCE study, which had just been published, prompted closure of the study. This study and its discontinued status were originally discussed at the 2004 ODAC meeting.

5.2.2.2 EPO-GBR-7

J&JPRD Study EPO-GBR-7 was a randomized, controlled, open-label, Phase 3, multicenter study. The primary objective was to evaluate the effect of treatment with epoetin alfa on the length of local disease-free survival, local tumor control, and patient-reported outcomes in subjects receiving radical radiotherapy with curative intent for head and neck cancer. The study was stopped in 2002 due to slow accrual, with only 301 of the planned 800 subjects having been enrolled. Subjects were randomly assigned to receive either standard radiotherapy plus epoetin alfa (Eprex) (4,000 or 10,000 IU SC 3 times per week based on whether entry hemoglobin concentration was >12.5 g/dL or ≤12.5 g/dL) or standard radiotherapy alone. The duration of treatment was through the end of radiotherapy. Subjects were to have a baseline hemoglobin concentration of less

than or equal to 15 g/dL. Hemoglobin concentration was to be maintained at approximately 12.5 g/dL to 15 g/dL.

The study's 5-year follow-up phase is ongoing. Epoetin alfa treatment had no effect on local tumor responses assessed 12 weeks after completion of radiotherapy. Similar results were seen for complete or partial response to radiotherapy on the lymph nodes (96% in both groups).

Local tumor evidence was assessed at weeks 1, 4, and 8 after radiotherapy, and at years 1, 2, 3, and 5 during the follow-up period. Based on the data available at this time, epoetin alfa treatment appeared to have no effect on the outcomes of these assessments. Similarly, epoetin alfa treatment did not have an apparent effect on local tumor recurrence within the irradiated volume at study completion or discontinuation (29% versus 25% of subjects in the observation and epoetin alfa groups) or relapse outside the irradiated volume (15% versus 13% of subjects in the observation and epoetin alfa groups).

At the time of the last update, 57 subjects (38%) in both the observation group and epoetin alfa group were known to have died. At the time of the last analysis, the Kaplan-Meier estimate of the 1-year survival rate was 79.9% for the observation group and 77.3% for the epoetin alfa group, yielding a difference of -2.6% (epoetin alfa versus observation) with a 95% confidence interval of (-12.08% to 6.8%). The difference between the treatment groups was not statistically significant (p = 0.867, log rank test).

A total of 2 subjects (1%) in the observation group and 5 subjects (3%) in the epoetin alfa group were reported to have experienced at least 1 TVE. Three of the TVEs (cardiac arrest, myocardial infarction, and embolism pulmonary) were clinically relevant and were reported in epoetin alfa-treated subjects. This study was originally discussed at the 2004 ODAC meeting.

5.2.2.3 Conclusions: Head and Neck Cancer

All 4 studies (ENHANCE, DAHANCA 10, RTOG 99-03, and EPO GBR-7) had as their primary endpoints locoregional control (progression-free survival or failure) and overall survival. All 4 studies were conducted with target hemoglobin concentrations beyond those recommended in the prescribing information. In the ENHANCE study, the control group had significantly better locoregional progression-free survival and overall survival compared with the epoetin alfa treatment group. Similar results were also noted in the

interim analysis of DAHANCA 10. In the remaining 2 studies, there was no statistically significant difference in either local regional failure or in overall survival. Based on these data, we conclude that the currently available evidence does not support the use of ESAs administered to target higher hemoglobin levels as an adjunct to therapeutic head and neck radiation. This important risk information is highlighted in the boxed warning of the current prescribing information.

5.2.3 Other Radiotherapy Indications With High Hemoglobin Targets

5.2.3.1 Cervical Cancer - Study AGO/NOGGO10/GER-8

This investigator-sponsored, Phase 3, multicenter, randomized, open-label study (referred to as Study AGO/NOGGO [Gynecology, Obstetrics Working Group/Northeast German Society for Gynecology and Oncology]) was conducted in subjects with high-risk cervical cancer receiving radiotherapy or sequential adjuvant chemotherapy followed by radiotherapy with or without epoetin alfa. Following radical hysterectomy, subjects were randomly assigned to 1 of 3 groups: adjuvant radiotherapy only (radiotherapy group), adjuvant chemotherapy plus radiotherapy (control group), or adjuvant chemotherapy plus radiotherapy and epoetin alfa (epoetin alfa group). The primary end point of the study was to evaluate relapse-free survival (or disease-free survival) after 5 years. Secondary endpoints included the change in hemoglobin concentrations, transfusion requirements, reduction in anemia, patient-reported outcomes, and overall survival.

At baseline (after surgery but before chemotherapy or radiotherapy) subjects in the epoetin alfa group began treatment with 10,000 IU epoetin alfa 3 times weekly, SC. Administration of epoetin alfa continued until 3 weeks after the end of radiotherapy to achieve a target hemoglobin concentration of 13 g/dL. Epoetin alfa was discontinued if hemoglobin concentration was >14 g/dL. Subjects in both the control group as well as the epoetin alfa group received transfusions if their hemoglobin concentrations declined to < 9 g/dL.

Assessment of relapse-free survival at 64.5 weeks (median observation time) showed that twice as many subjects in the control group (22%) as in the epoetin alfa group (11%) had recurrence ($p = 0.04$). The difference in recurrence between the groups at the 105-week observation was smaller (25% versus 17% for the control and epoetin alfa groups, respectively), but trended toward significance ($p = 0.074$) (Blohmer JU, unpublished data, 2006).

Although significant differences were noted in anemia between the epoetin alfa group and the control group, there were no significant differences between the study groups with respect to indicators of hematologic or non-hematologic toxicity. Results on overall survival are as yet incomplete because the study remains open to follow-up of the surviving subjects.

5.2.3.2 Gastric or Rectal Cancer - Study PR00-03-006

This was a double-blind, placebo-controlled, multicenter study in subjects with gastric or rectal cancer receiving fluoropyrimidine chemotherapy concurrently with radiation. The study was intended to assess transfusion requirements, hematologic effects, patient-reported outcomes endpoints, and tumor response. Up to 184 subjects were to be randomized 1:1 to receive epoetin alfa 40,000 IU SC once weekly or placebo. Subjects had hemoglobin levels of ≥ 10 to < 15 g/dL at entry, and were treated with Procrit at 40,000 IU/week, with dose adjustments depending on response. The study was stopped based on the recommendation of a Data Safety Monitoring Board based on an imbalance in the number of subjects who experienced deep vein thrombosis in the 2 treatment groups.

Data were available for 59 subjects at the time the study was analyzed. Eight subjects experienced at least 1 TVE, 2 of 31 subjects (6%) treated with placebo and 6 of 28 subjects (21%) treated with epoetin alfa. Seven of 8 TVEs were deep vein thromboses and were assessed by the investigator as serious. The eighth TVE was chest pain and was assessed as not serious. TVEs occurred in 6 of 53 subjects (11%) with rectal cancer and 2 of 6 subjects (33%) with gastric cancer.

Seven of 35 subjects (20%) with baseline hemoglobin > 13 g/dL experienced at least 1 TVE, compared with 1 of 24 subjects (4%) with a baseline hemoglobin ≤ 13 g/dL. Patients commonly had a hemoglobin level > 13 g/dL within the 28 days before the TVE; but such levels were also common in patients who did not have TVEs. No patient had a hemoglobin increase of more than 2 g/dL in the 4-week period before the occurrence of a TVE.

Due to the early termination of the study, only descriptive statistics were provided for efficacy endpoints. A total of 10 subjects (32%) in the placebo group and 4 subjects (14%) in the epoetin alfa group received RBC transfusion, and both groups had similar changes from baseline in QOL to last value (final) observed. This study and its discontinued status were originally discussed at the 2004 ODAC meeting.

5.2.3.3 Cervical Cancer – Study PR01-04-005/GOG-0191

This was an open-label, randomized, multicenter, investigator-sponsored study in subjects with cervical cancer receiving concurrent radiation and cisplatin. The study was intended to determine whether epoetin alfa treatment to maintain higher hemoglobin levels could prolong progression-free survival (primary clinical endpoint). Secondary clinical end points included overall survival and local tumor control. Planned recruitment was 460 subjects. Subjects were randomized 1:1 to receive epoetin alfa 40,000 IU SC once weekly or standard of care. Eligible subjects had hemoglobin concentrations < 14 g/dL at entry. Dosing was interrupted if hemoglobin exceeded 14 g/dL for 2 weeks or more, and then was restarted at the same dose when hemoglobin fell to <13 g/dL. The study was discontinued because an analysis of preliminary data showed a higher than expected occurrence of TVEs.

Data were available for 113 subjects at the time the study was analyzed. Fifteen subjects experienced at least 1 TVE: 5 of 55 subjects (9%) receiving cisplatin+radiation and 10 of 58 subjects (17%) receiving cisplatin+radiation+epoetin alfa. TVEs were classified as venous in 10 subjects, arterial in 3 subjects and were unclassifiable in 2 subjects. There was no apparent association between level of hemoglobin at baseline or on treatment and the occurrence of a TVE.

Two subjects, 1 in each treatment arm, died during the study or within 30 days following the last dose of study drug. Overall 9 subjects (16%) in the cisplatin+radiation group and 8 subjects (14%) in the cisplatin+radiation+epoetin alfa group died during the study treatment and the follow-up period (up to approximately 26 months). Survival and progression-free survival were similar for the 2 treatment groups, and the disease recurrence and progression rate was 18% for the cisplatin+radiation group and 17% for the cisplatin+radiation+epoetin alfa group.

Four subjects receiving cisplatin+radiation+epoetin alfa had a hemoglobin increase of more than 2 g/dL in the 4-week period prior to the TVE. These hemoglobin increases could be explained by packed RBC transfusions for 3 of the 4 subjects. This study and its discontinued status were originally discussed at the 2004 ODAC meeting.

5.2.3.4 Non-small-cell Lung Cancer: EPO-GER-22

Study EPO-GER-22 was a Company-sponsored, phase 3, prospective, randomized, controlled, multicenter open-label study in patients with Stage III non-small cell lung

cancer. The study was to enroll 612 subjects in a 1:1 ratio to standard chemotherapy (50 mg/m^2 cisplatin and 30 mg/m^2 vinorelbine by intravenous infusion) or standard chemotherapy plus epoetin alfa 40,000 IU SC once weekly. In addition, all subjects were to receive daily radiotherapy (total dose 66 Gy: 2 Gy/day, 5 times/week) from Day 64 to Day 108. As originally designed, subjects assigned to receive epoetin alfa could start to receive study drug when their hemoglobin concentration fell below 13.0 g/dL. Study drug was withheld at when hemoglobin exceeded 14 g/dL and could be resumed when hemoglobin fell below 13 g/dL. The protocol was amended in October 2003 to require that subjects have a hemoglobin concentration < 12 g/dL to start treatment, with treatment withheld at concentrations exceeding 13 g/dL, and only resumed again when hemoglobin fell below 12 g/dL.

The primary endpoint was the 2-year survival rate. Secondary endpoints included tumor remission rate after chemotherapy treatment, local tumor control, patient-reported outcomes, hemoglobin concentrations, transfusion rates, and the safety and tolerability of epoetin alfa.

As of December 2005, a total of 389 subjects had been enrolled in 39 centers. Interim data are available for 215 subjects (108 epoetin alfa, 107 control) as of November 2004. Data for all subjects, whether enrolled before or after the protocol amendment, were combined for all variables analyzed. There were no differences between groups with respect to demographics, chemotherapy, or radiation therapy.

Two-year survival data are not available at this time because the majority of the subjects have not completed the follow-up period.

When all subjects were analyzed regardless of baseline hemoglobin concentrations, there was no evidence of a survival disadvantage in subjects who received epoetin alfa compared with subjects who did not, although data are still incomplete. At the time of this analysis, 56% of subjects were alive and the total number of deaths was similar between the 2 groups (epoetin alfa [n = 48], control [n = 47]). The median survival for subjects who received epoetin alfa was 338 days (95% CI: 242, 434) versus 299 days (95% CI: 234, 364) for subjects who did not receive epoetin alfa.

A post hoc analysis of data from subjects with baseline hemoglobin concentrations <13 g/dL showed no evidence of a survival disadvantage in subjects who received epoetin alfa compared with subjects who did not. Median survival for subjects who

received epoetin alfa was 385 days (95% CI: 208 to 562) versus 227 days (95% CI: 170 to 284) for subjects who did not. Post hoc analysis of data from subjects with baseline hemoglobin concentrations ≥ 13 g/dL showed that the median survival was less in subjects who received epoetin alfa compared with subjects who did not. Median survival for subjects who received epoetin alfa was 265 days (95% CI: 139 to 391) versus 391 days (95% CI: 222 to 560) for subjects who did not. To date, the number of deaths within each group is too small to determine whether the difference between groups for these analyses are clinically meaningful.

At the time of the most recent interim analysis, twice as many subjects who received epoetin alfa were reported to have experienced at least 1 TVE compared with subjects who did not receive epoetin alfa, although this difference was not significant (p = 0.097). When TVE occurrence was analyzed in subjects with baseline hemoglobin concentrations ≥ 13 g/dL, more epoetin alfa-treated subjects (15 [23%] of 6 subjects) experienced TVEs compared with control subjects (6 [9%] of 67 subjects). Alternatively, when TVE occurrence was analyzed in subjects with baseline hemoglobin concentrations < 13 g/dL, 5 (11%) of 44 epoetin alfa-treated subjects experienced TVEs compared with 4 (10%) of 40 control subjects.

5.2.3.5 Conclusions: Other RT Indications

Due to the early termination of the Gastric or Rectal Cancer Study (PR00-03-006) and the Cervical Cancer Study (PR01-04-005/GOG-0191) no conclusions can be made regarding the impact of epoetin alfa therapy on disease outcomes based on these 2 studies. In both studies, which utilized high hemoglobin targets, epoetin alfa-treated subjects had increased TVE occurrence compared with control-group subjects. Preliminary results from the Cervical Cancer Study (AGO/NOGGO10/GER-8) show no signal of decreased survival, or progression free survival in epoetin alfa-treated subjects. Recurrence at the 105-week observation was less frequent for epoetin alfa group subjects than control group subjects (17% versus 25% respectively) trending toward significance (p = 0.074) (Blohmer JU, unpublished data, 2006). At an interim analysis of a study of patients with inoperable non-small cell lung cancer, mortality was similar in the epoetin alfa and control groups. Follow up in this study is continuing.

6. Assessment of Risk/Benefit of ESAs in Oncology

Chemotherapy-induced Anemia

Based upon the totality of the available data, including the original pivotal studies for licensing, additional randomized placebo-controlled studies, meta-analyses of both patient-level and study-level data from Amgen-sponsored clinical trials, and meta-analyses of patient- and study-level data from J&JPRD sponsored clinical trials, no evidence of an increased risk of disease progression or death with ESAs relative to placebo was observed for subjects when treated within the labeled indication. As expected, a higher risk of CV/TE events was observed for ESAs relative to placebo, primarily driven by an increased rate of embolism/thrombosis. ESAs were associated with a significantly lower rate of transfusions and a significantly greater rate of hematopoietic response compared with placebo in subjects with CIA. Based on the evidence from these comprehensive analyses, Amgen and J&JPRD believe that the risk/benefit of ESAs remains favorable when administered per the label to the indicated patient population (CIA), and that the risks are adequately addressed in the current label information.

Anemia of Cancer

An increased risk of death relative to placebo was observed in a recent phase 3 study in anemic subjects with active cancer treated with darbepoetin alfa who were not receiving chemotherapy or radiotherapy (Study 20010103). The patients enrolled in this study represent a different population than the patients with CIA. In this study, the patients that were enrolled had completed all of their therapeutic options and had a poor prognosis as verified by the high mortality in this study. Patients treated for CIA, in contrast, are receiving treatment and typically have a better prognosis. The etiology of the increased risk of death in the patients studied in Study 20010103 is unclear, and these results are discordant with the neutral impact of survival in patients treated with ESAs for CIA, as characterized by the meta-analyses provided in Section 4.2 and 5.1. The increased risk of death in cancer patients treated with ESAs who are not receiving chemotherapy or radiation therapy has been communicated in the boxed warning in the prescribing information for darbepoetin alfa and epoetin alfa, and through a Dear Health Care Professional letter by Amgen and J&JPRD.

Head and Neck Cancer

Use of ESAs to target higher hemoglobin concentrations in an attempt to enhance tumor oxygenation in patients with head and neck cancer receiving radiation therapy may result in poorer loco-regional control compared with placebo (ENHANCE, DAHANCA 10). Use of ESAs in this setting is investigational and, based on the data in DAHANCA 10, should be avoided. The nature of this risk is not well defined, since the methods used to define locoregional control are not well validated as a measure of tumor progression. The results of ENHANCE were communicated in previous product labeling and the DAHANCA study is also cited in the recently updated labeling.

7. Ongoing Risk Management Plan and Pharmacovigilance Program for Darbepoetin alfa and Epoetin alfa in Oncology

7.1 Risk Minimization and Communication

As part of the ongoing risk management plans for darbepoetin alfa and epoetin alfa, Amgen and J&JPRD have addressed safety concerns through product labeling updates and risk communications, including Dear Health Care Professional letters.

After the 2004 ODAC meeting, the identified risks associated with ESAs in oncology were addressed in the prescribing information for darbepoetin alfa and epoetin alfa, as discussed in Section 2.4. Based on recent safety data outlined in Section 2.5, the following additional actions were taken.

On 26 January 2007, Amgen communicated the results of Amgen Study 20010103 in a Dear Health Care Professional letter. This letter described the increased mortality observed in the darbepoetin alfa group relative to placebo in subjects with active cancer not receiving chemotherapy or radiotherapy, an unapproved patient indication.

On 9 March 2007, a BOXED WARNING was added to the prescribing information for all ESAs. This warning addressed the recent study findings discussed in Section 2.5.

Statements identifying the increased risk of death and serious cardiovascular events in cancer (as well as in chronic kidney disease) patients treated with ESAs when administered to target a hemoglobin of greater than 12 g/dL were included. In addition, at the request of FDA, the following statements were also added to the BOXED WARNING concerning the potential for tumor progression:

Cancer Patients: Use of ESAs:

- *shortened the time to tumor progression in patients with advanced head and neck cancer receiving radiation therapy when administered to target a hemoglobin of greater than 12 g/dL;*
- *shortened overall survival and increased deaths attributed to disease progression at 4 months in patients with metastatic breast cancer receiving chemotherapy when administered to target a hemoglobin of greater than 12 g/dL;*
- *increased the risk of death when administered to target a hemoglobin of 12 g/dL in patients with active malignant disease receiving neither chemotherapy nor radiation therapy. ESAs are not indicated for this population.*

(See WARNINGS: Increased Mortality and/or Tumor Progression)

Summary data were also included in the WARNINGS section of the prescribing information, referring to data obtained on the risk of tumor progression and survival in certain patient populations.

At the request of FDA, the DOSAGE AND ADMINISTRATION section was also amended to advise physicians that the dose of ESAs should be adjusted for each patient to maintain the lowest hemoglobin level sufficient to avoid the need for RBC transfusion and not to exceed 12 g/dL.

On 12 March 2007, Amgen and J&JPRD informed healthcare professionals about the revisions to the US prescribing information through a joint Dear Health Care Professional letter.

Amgen and J&JPRD continue to evaluate the adequacy of the current prescribing information and other means of risk communication to alert healthcare professionals and patients to the importance of identified and potential risks.

7.2 Amgen's Postmarketing Status: Ongoing Pharmacovigilance Studies of Darbepoetin alfa

The Aranesp Pharmacovigilance Program includes 5 randomized, prospective clinical studies designed to evaluate specific cancer endpoints in a variety of malignancies (Table 11). These clinical trials include both investigator-sponsored studies (FR-2003-3005, DE-2001-0033, DE-2002-0015, and SE-2002-9001) and an Amgen-sponsored study (20010145).

As discussed previously, these 5 trials were ongoing at the time of the 04 May 2004 ODAC meeting. Subsequent to discussions with FDA following the ODAC meeting in 2004, FDA accepted reporting the results of these studies as formal post-marketing commitments. The intent is that data from these studies will serve as a means of prospectively addressing disease progression and survival outcomes in patients receiving darbepoetin alfa therapy. As an additional post-marketing commitment, Amgen will perform a meta-analysis of the 5 trials in the pharmacovigilance program.

The investigator-sponsored trials are open-label, randomized studies of darbepoetin alfa versus observation, whereas the Amgen-sponsored study (20010145) is randomized, double-blind, and placebo-controlled. Three of the trials address relevant tumor and

survival endpoints in breast cancer (DE 2001 0033, DE-2002-0015) and head and neck cancer (DAHANCA 10) in conjunction with chemotherapy and radiotherapy, the settings in which the adverse outcomes in the BEST (Leyland-Jones, 2003) and ENHANCE (Henke et al, 2003) trials were noted, which were the primary studies warranting the May 2004 ODAC meeting. Two other tumor types are also included in the pharmacovigilance program; small-cell lung cancer (Amgen-sponsored study 20010145) and non-Hodgkin's lymphoma (FR-2003-3005). In all studies, endpoints include various outcomes relating to disease progression and survival, such as event-free survival, relapse, overall survival, and locoregional control.

Table 11. Aranesp Pharmacovigilance Program

Study Designation(s) (Sponsor/Institute)	Study Title
20010145 (Amgen)	A randomized, double blind, placebo-controlled study of subjects with previously untreated extensive-stage small-cell lung cancer (SCLC) treated with platinum plus etoposide chemotherapy with or without darbepoetin alfa
FR-2003-3005 LNH03-6B (GELA)	Randomized study of intensified CHOP plus rituximab given every 14 days (R-CHOP 14) versus CHOP plus rituximab given every 21 days (R-CHOP 21) and randomized study of frontline/prophylactic darbepoetin alfa treatment versus usual symptomatic treatment of anemia in non previously treated patients aged from 60 to 80 years, with CD20+ diffuse large B-cell lymphoma
DE-2001-0033 PREPARE (AGO)	Randomized comparison of a preoperative, dose-intensified, interval-shortened sequential chemotherapy with epirubicin, paclitaxel and CMF ± darbepoetin alfa versus a preoperative, sequential chemotherapy with epirubicin and cyclophoshamide followed by paclitaxel in standard dosage ± darbepoetin alfa in patients with primary breast cancer
DE-2002-0015 ARA 03 ARA PLUS (WSG)	Adjuvant therapy for breast cancer: Impact of erythropoiesis-stimulating factors on survival in high-risk breast cancer treatment. Prospective randomized comparison of CEF/TAC chemotherapy ± darbepoetin alfa (Aranesp®) for patients with positive lymph nodes
SE-2002-9001 DAHANCA 10 (DAHANCA)	Study of the importance of novel erythropoiesis stimulating protein (Aranesp®) for the effect of radiotherapy in patients with primary squamous cell carcinoma of the head and neck

All 5 trials include planned interim safety analyses to ensure careful monitoring, and are being conducted with oversight by independent DMCs or data safety monitoring boards (DSMB) that will review both efficacy and safety data and make determinations regarding the continuation of the studies.

Information relating to the status of the studies, which has been obtained from the respective principal investigators and/or the DMCs since the May 2004 ODAC, is summarized within the following sections.

7.2.1 Amgen Study 20010145: Small-cell Lung Cancer

Study 20010145 is a randomized, double-blind, placebo-controlled study to evaluate the effects on survival of increasing or maintaining hemoglobin with darbepoetin alfa in anemic (hemoglobin > 9.0 g/dL and < 13.0 g/dL) subjects receiving chemotherapy for previously untreated, extensive-stage small-cell lung cancer. Darbepoetin alfa was administered at a dose of 300 μg QW for 4 weeks followed by 300 μg Q3W for the remainder of the treatment period. Darbepoetin alfa was withheld for hemoglobin concentrations ≥ 14.0 g/dL until concentrations declined below 13.0 g/dL. Approximately 600 subjects were enrolled and were followed until 496 deaths had occurred; the remaining 104 subjects will be followed until death.

The co-primary endpoints for this study are change in hemoglobin from baseline to the end of the chemotherapy treatment period and survival time, which are to be tested in a step-down manner. Survival time will be tested if the mean change in hemoglobin concentration endpoint is statistically significant (at the 0.0463 level for the final analysis). In addition, the safety of darbepoetin alfa will be further evaluated based on the incidence and severity of adverse events, changes in laboratory results, changes in vital signs, and the incidence of concomitant medication use.

The protocol-specified number of events based on its event-driven design has been met. At this time, during the final data reconciliation period, Amgen remains blinded to the information.

Two interim analyses reviewed by the DMC occurred during the course of this study, with decision by the DMC at each analysis to continue the study. These interim analyses included subject-level unblinding for DMC members. Amgen remained blinded to the subject-specific information.

Study 20010145 is unique within the current pharmacovigilance program, as it is the only randomized, double-blind, placebo-controlled study prospectively designed to evaluate long-term survival in subjects with CIA, albeit at a somewhat higher hemoglobin target than that in the current US label. However, the study was conducted in accord with

target hemoglobin approved in the European Summary of Product Characteristics (SPC) current at the time of study start.

At the time of finalizing this briefing document, the Aranesp oncology team remains blinded to the data from this study, though it is anticipated that results will become available prior to the time of the ODAC meeting. As such, key clinical data will be available to help inform the medical community with regard to the re-evaluation of risk management for ESAs (see Section 7.5). The original projected completion date as per the agreed postmarketing commitment is 31 October 2007.

In addition, radiological material is currently undergoing blinded, central review and will subsequently be available to provide meaningful information regarding the issues of tumor response and tumor progression.

7.2.2 GELA LNH 03-6B (FR-2003-3005): Study in Diffuse Large B-cell Lymphoma

Study FR-2003-3005 is an open-label, randomized, multicenter, phase 3 investigator-sponsored trial being conducted in France by Groupe d'Etude des Lymphomes de l'Adulte (GELA). It is anticipated that 600 patients will be enrolled. This study evaluates the efficacy of rituximab plus CHOP chemotherapy given every 14 days (R-CHOP 14) compared with the standard R-CHOP 21 regimen in previously untreated patients aged 66 to 80 years with diffuse large B-cell lymphoma. The primary endpoint is event-free survival. Patients in each treatment group are further randomized to receive either frontline/prophylactic darbepoetin alfa or no prophylactic treatment. Darbepoetin alfa will be administered weekly for patients with hemoglobin concentrations < 13 g/dL at a dose of 100 μg for subjects < 60 kg, 150 μg for subjects between 60 and 80 kg, and 200 μg for subjects > 80 kg. Darbepoetin alfa doses are to be reduced at hemoglobin concentrations ≥ 14.0 g/dL and withheld at concentrations > 15.0 g/dL. Patients not randomized to darbepoetin alfa with symptomatic anemia have the opportunity to receive another ESA according to local practice. An interim analysis for safety and for the primary efficacy endpoint was planned after 2 years.

This study was initiated with a target hemoglobin level of 13 g/dL, which was consistent with the approved United States package insert at the time. Based on changes to the European Summary of Product Characteristics (SPC) that occurred while this study was underway reducing the target hemoglobin level to 12 g/dL, GELA was asked by the French regulatory body (AFSSAPS) to consider conforming to new dosing algorithms in

the regional package insert. As a result, the study group suspended randomization in order to analyze the first available data from the study. Based on the interim analysis, the DMC determined that minor modifications to dosing algorithms should be made in the trial. The protocol was thus amended to maintain hemoglobin levels between 11 and 13 g/dL, in accordance with the revised SPC.

Interim data from this study involving 134 patients were presented at the 2006 ASH meeting (Delarue et al, 2006). These interim results indicated that subjects receiving darbepoetin alfa required significantly fewer RBC transfusions compared to those receiving standard of care ($p = 0.01$). The median hemoglobin level during treatment was 12.05 g/dL in subjects who received darbepoetin alfa and 10.65 g/dL in those who did not. No negative impact of darbepoetin alfa on event-free or overall survival was observed at the time of the analysis. Overall survival at 1 year was 78% for darbepoetin alfa versus 70% for standard of care (RR 0.75, 95% CI: 0.44 – 1.76), and event-free survival at 1 year was 73% for darbepoetin alfa and 64% for standard of care (RR 0.75, 95% CI: 0.44 – 1.26). Amgen has been informed that the GELA study currently has their next planned DSMB meeting in May 2007. The original projected completion date as per the agreed post-marketing commitment is 31 August 2010.

7.2.3 AGO PREPARE (DE-2001-0033): Study in Neoadjuvant Breast Cancer

Study DE-2001-0033 is an open-label, randomized, multicenter, phase 3 trial conducted by the German Gynecological Oncology Study Group (AGO). The study was designed to evaluate the effects of preoperative chemotherapy using a sequential dose-dense and dose-intensified regimen of epirubicin, paclitaxel, and CMF compared with preoperative sequential administration of epirubicin and cyclophosphamide followed by paclitaxel in patients with breast cancer, with or without darbepoetin alfa. Darbepoetin alfa was administered at a dose of 4.5 μg/kg Q2W to maintain hemoglobin concentrations between 12.5 g/dL and 13 g/dL. The dose was doubled at the time of the third dose if the hemoglobin concentration has not increased by at least 1 g/dL from baseline and was withheld for hemoglobin concentrations $\geq$ 14.0 g/dL. A total of 720 subjects were planned for accrual into this study.

The primary endpoint of this study is the effect of dose-dense, dose-intense preoperative chemotherapy on relapse-free survival. Secondary endpoints are the effects of preoperative dose-dense, dose-intense preoperative chemotherapy on clinical and

pathological remission rates and the effects of darbepoetin alfa on remission rate and QOL. An interim analysis at 3 years was planned for safety and for the primary endpoint. This study included a data monitoring committee.

Accrual to this study is complete and follow-up continues. Due to logistical complications between the principal investigator, his current hospital affiliation, and a previous study sponsor, data collection has been stalled. Amgen is aware of one interim analysis and corresponding DMC data review for this study, where the recommendation was to continue the trial unchanged. Amgen is working with the principal investigator and the other involved parties with the goal of accelerating completion of the data acquisition and analysis. The original projected completion date as per the agreed postmarketing commitment is 30 November 2007.

7.2.4 WSG ARA-03 (DE-2002-0015): Study in Adjuvant Breast Cancer

Study DE-2002-0015 is an open-label, randomized, multicenter, phase 3 trial conducted by the West German Study Group (WSG). The study is designed to evaluate the effects of adjuvant chemotherapy with and without darbepoetin alfa on event-free survival rates in patients with breast cancer who have positive lymph nodes. One thousand two hundred thirty-four patients will be accrued to this study. Patients will receive local radiotherapy at the completion of chemotherapy. Darbepoetin alfa will be initiated in subjects with hemoglobin concentrations ≤ 13.5 g/dL at a dose of 300 μg weekly for 4 weeks followed by 300 μg Q3W. Dosing will be interrupted for hemoglobin concentrations > 14 g/dL until the hemoglobin value decreases to 13.5 g/dL, at which time dose will resume at 300 μg every 3 weeks.

The primary endpoint is event-free survival, defined as relapse (local or distant), deaths from any cause, or second primaries. The secondary endpoints are overall survival, local relapse rate, toxicity, cognitive function, and severity of patient-reported anemia symptoms.

The study remains ongoing. During the course of the study, the study protocol was amended to remove the loading dose of QW for weeks 1-4 with a Q3W dose for the entire treatment period, as well as to reduce the target hemoglobin level from ≤ 13.5 g/dL to ≤ 13.0 g/dL. One interim safety analysis has been reviewed by the study DMC, with the recommendation that the study continue. The original projected completion date as per the agreed postmarketing commitment is 31 May 2011.

7.2.5 DAHANCA 10 (SE-2002-9001): Study in Head-and-Neck Cancer

This study was an open-label, randomized, multicenter, phase 3 investigator-initiated study conducted by the Danish Head and Neck Cancer Study Group (DAHANCA). The study was designed to evaluate the effects of darbepoetin alfa in patients with head-and-neck cancer receiving primary curative radiotherapy. Planned enrollment for this study was 600 patients. Darbepoetin alfa was administered at a dose of 150 μg weekly for subjects with hemoglobin concentrations < 14 g/dL. If the hemoglobin concentration was ≥ 14 g/dL and ≤ 15 g/dL, subjects received 80 μg weekly. Darbepoetin alfa was to be withheld for hemoglobin concentrations > 15.5 g/dL. If, after 4 weeks, the hemoglobin concentrations were declining, darbepoetin alfa was to be increased to 300 μg weekly. The clinical hypothesis was that the addition of darbepoetin alfa to standard curative radiotherapy treatment of head-and-neck cancer would increase the locoregional disease control rate.

The primary endpoint of this study was locoregional control (T+N localization). Secondary endpoints include local control (T-localization), overall survival, disease-specific survival, and hemoglobin concentrations during radiotherapy, and acute toxicity. A formal interim analysis was planned after the observation of 150 locoregional failures. The original projected completion date as per the agreed postmarketing commitment is 30 September 2008. Amgen received the first formal written notification of interim study data from the study group on 01 December 2006, with the investigators notified concurrently. The results that Amgen was informed of at that time represented preliminary data from an interim analysis that occurred after the trial was temporarily stopped on 18 October 2006, following publication of the post-hoc analysis of immunohistochemical data on the putative expression of the EpoR from the Henke head and neck cancer trial (Henke et al, 2003; Henke et al 2006).

As noted in Section 2.5, the preliminary interim report by the principal investigator shows an approximate 10% difference in 3-year locoregional control ($p = 0.01$) in favor of the control group. Overall survival showed a smaller nonsignificant difference in favor of the control group ($p = 0.08$). No differences in the rate of distant metastasis or death from non-cancer causes have been identified. Treatment with darbepoetin alfa has not been associated with any excess serious adverse events in this preliminary interim analysis. While locoregional control is the primary endpoint of the study, this endpoint (in the absence of overall survival data), and particularly the process whereby it was evaluated in this study, is considered to be subjective in nature. As such, particularly in an open-

label study, care must be paid to evaluating such events. At present, it is unclear to Amgen as to what extent data monitoring / verification has occurred and whether there have been any data audits. Amgen will continue to work with the study group to better understand these considerations and any ramifications they may have on the interpretation of results.

Based on this preliminary interim data, the DAHANCA group concluded that the trial would be highly unlikely to demonstrate improved outcomes with darbepoetin alfa treatment and decided to terminate enrollment into the study.

Amgen continues to foster open communication with the principal investigator and has offered financial assistance to enable collation and analysis of the data. Amgen has received no additional written information or data on DAHANCA 10 study results from the principal investigator since the original written communication provided on 01 December 2006. This information is consistent with the information in the public domain as provided on the DAHANCA web site.

7.2.6 Statistical Considerations for Amgen's Pharmacovigilance Program

As presented in Amgen's May 2004 ODAC briefing document, a summary of the power and sensitivity of the Aranesp Pharmacovigilance Program is provided in Table 12, along with updated accrual information. Individually, these trials have 80% power to rule out an increase in risk of 32% to 53% for disease progression and of 42% to 70% for survival with darbepoetin alfa.

As noted previously, Amgen will perform 2 meta-analyses of the 5 studies mentioned above. The original projected completion date as per the agreed post-marketing commitment is 31 December 2011.

These meta-analyses will be performed to increase the statistical power and sensitivity of the individual pharmacovigilance studies. The first analysis will combine all 5 studies, resulting in 80% power to detect a hazard ratio of 1.15 or greater. The second analysis will combine the 2 breast cancer studies (PREPARE and ARA-03), resulting in 80% power to detect a hazard ratio of 1.3 or greater.

Table 12. Aranesp Pharmacovigilance Program Trials: Power and Sensitivity Calculations

				Progression Sensitivity 80% Power		Survival Sensitivity 80% Power	
Study Designation(s) (Sponsor/Institute)	Tumor Type	Design	Accrual/Planned March 2007	%	Hazard Ratio	%	Hazard Ratio
20010145 (Amgen)	Small cell lung	Cisplatin / Carboplatin/VP16 ± Aranesp	600/600	EFS 25% ± 9% at 6 months	1.32	50% ± 10% at 6 months	1.42
FR-2003-3005 LNH 03-6B (GELA)	Non-Hodgkin's lymphoma	R-CHOP 14 or R-CHOP 21 ± Aranesp	370/600	EFS 55% ± 11% at 3 years	1.45	65% ± 11% at 3 years	1.53
DE-2001-0033 PREPARE (AGO)	Breast	Sequential or dose-intensified chemotherapy ± Aranesp	735/720	RFS 70% ± 10% at 5 years	1.53	80% ± 10% at 5 years	1.70
DE-2002-0015 ARA 03 ARA PLUS (WSG)	Breast	Adjuvant chemotherapy ± Aranesp	801/1234	EFS 60% ± 9% at 5 years	1.35	75% ±7% at 5 years	1.48
SE-2002-9001 DAHANCA 10 (DAHANCA)	Head and neck	Radiotherapy ± Aranesp	531/600	Local Control 50% ± 11% at 5 years	1.42	60% ± 11% at 5 years	1.49

± indicates detectable difference; EFS = event-free survival; RFS = relapse-free survival

7.3 J&JPRD Postmarketing Status: Ongoing Pharmacovigilance Studies of Epoetin alfa

Following the 2004 ODAC meeting, J&JPRD agreed to provide the FDA with periodic safety updates from 5 ongoing, randomized Eprex studies that included survival as an endpoint (EPO-GBR-7, EPO-GER-22, AGO/NOGGO, EPO-CAN-17, and AGO adjuvant breast study [the Möbus Study]) (Table 13). These updates have been provided previously to the FDA (Jameson, 2004). Brief descriptions of currently available information for Study EPO-GBR-7, AGO/NOGGO, and EPO-GER-22 have been provided in Section 5.1.1.

An attempt was made to investigate tumor progression in the original postmarketing commitment study, Study N93-004, a double-blind, placebo-controlled study designed to enroll subjects with newly-diagnosed limited or extensive stage small cell lung cancer who were to be treated with etoposide and cisplatin. This commitment was discharged in May 2004 (see Section 7.3.1 for more information). At that time, J&JPRD committed to investigate the risk/benefit profile of epoetin alfa in Study EPO-ANE-3010, entitled "A Randomized, Open-Label, Multicenter, Phase 3 Study of epoetin alfa Plus Standard Supportive Care Versus Standard Supportive Care in Anemic Patients with Metastatic Breast Cancer Receiving First-Line Standard Chemotherapy." The study was further refined with the objective of enhancing feasibility of timely completion after feedback from an expert external global Advisory Board in July 2004. The revised protocol was submitted to FDA in December 2004.

It should be noted that the above studies, with the exception of Study EPO-ANE-3010, have been conducted with the goal of treating subjects above the hemoglobin levels recommended in the prescribing information.

Table 13. Update of Open-Label, Randomized, Controlled EPREX Oncology Studies as of 31 March 2006

Study (Country) (Sponsor)	Type of Tumor	Study Design	First/Last Date of Subject Enrollment	Subjects Enrolled/ Planned	Primary End Point (s)	Interim Data/DSMB	Final Data
EPO-GBR-7 (Great Britain) (J&JPRD)[a]	HNC	Phase 3 study to evaluate the effect of epoetin alfa (4,000 or 10,000 IU, t.i.w., s.c. based on whether Hb was >12.5 g/dL or ≤12.5 g/dL) plus standard RT or standard RT alone.	Aug 1999/ Apr 2002 (enrollment closed)	301/800	Local DFS and Hb effect on local tumor control	10 Jun 2004	2007
EPO-GER-22 (Germany) (Ortho-Biotech Germany)	NSCLC	Prospective study to evaluate subjects undergoing sequential CT and RT to evaluate epoetin alfa therapy (40,000 IU, q.w., s.c. if Hb <13 g/dL) versus no epoetin alfa therapy.	Aug 2001/ Dec 2005 (enrollment closed)	389/612	2-year survival	Interim report: 28 Feb 2005; Final DSMB: 19 Mar 2005	2007 (Final report in 1-2Q08)
EPO-CAN-17 (Canada) (Ortho-Biotech Canada)	Breast	Phase 3b study to compare the effect of epoetin alfa (40,000 q.w., s.c) vs. SOC on Hb maintenance and QoL in subjects receiving CT for a maximum of 28 weeks.	2 Feb 2002/ 22 May 2003 (study initiation/ termination)	354/350	QoL	Analysis complete	2005 (Final report in March 2006)
Moebus (Germany) (Investigator-initiated)	Breast	Phase 3 study in subjects with node-positive breast cancer (≥4 nodes) to compare a dose-dense CT regimen of ETC + G-CSF (with and without epoetin alfa (150 IU/kg t.i.w.) with a conventional CT regimen (no epoetin alfa) on DFS, hematologic toxicity, and transfusion requirements.	Dec 1998/ Oct 2002	1284/1284 (593 randomized to dose-dense regimen)	2-year DFS, reduction in transfusion, difference in median Hb concentrations	Oct 2002/ Poster: San Antonio Breast Cancer Symposium (2003)	Study closed in 2005 – no further data expected.
AGO/NOGGO (Germany) (Investigator-initiated)	Cervical	Phase 3 study to evaluate the optimization of postoperative treatment in subjects with high risk of relapse. Treatment consists of RT alone, CT + RT, and epoetin alfa (10,000 IU, t.i.w.) + RT + CT.	Jan 1999/ Mar 2001	264/264	5-year DFS	Abstract: ECCO Symposium (2003)	2006

NOTE: J&JPRD=Johnson & Johnson Pharmaceutical Research & Development; QoL = quality of life; RT = radiotherapy; CT = chemotherapy; HNC = head and neck cancer; NSCLC = non-small cell lung cancer; q.w. = once weekly; t.i.w. = 3 times a week; DFS = disease-free survival; DSMB = Data Safety Monitoring Board; Hb = hemoglobin; s.c. = subcutaneous; ETC = epirubicin, paclitaxel and cyclophosphamide given at 2-week intervals; G-CSF = granulocyte colony-stimulating factor; SOC = standard of care; AGO/NOGGO = Gynecology, Obstetrics Working Group/Northeast German Society for Gynecology and Oncology; ECCO = European Conference on Clinical Oncology

[a] Sponsorship of this study was transferred from Ortho-Biothech Great Britain to J&JPRD in 2005.

7.3.1 Study N93-004: Study in Small-cell Lung Cancer

As noted previously, Study N93-004 was a double-blind, placebo-controlled study designed to address a Phase 4 commitment to enroll 400 subjects with newly-diagnosed, limited or extensive stage, small-cell lung cancer who were to be treated with etoposide and cisplatin. It was requested by the FDA to evaluate the possible stimulatory effects of epoetin alfa on solid tumor growth. This study was conducted in predominantly non-anemic cancer patients and was designed to assess tumor response, with survival as a secondary endpoint. Median survival time and overall survival were similar in the 2 treatment groups. In both arms of the study, tumor response and survival through Month 12 appeared similar. Beyond Month 12, there was divergence in the survival curves favoring the placebo group, though the data are sparse and complete follow-up information is not available. Although the results of Study N93-004 did not suggest any substantive effect of epoetin alfa on tumor treatment response or disease progression in SCLC and the 95% confidence intervals excluded an impairment of response rate of 6% or higher, the study was terminated for poor accrual in agreement with FDA after 224 subjects had been enrolled. As a consequence, the FDA viewed the study to be non-definitive and requested a new commitment study (see Section 7.3.2).

7.3.2 Study EPO-ANE-3010: Study in Metastatic Breast Cancer

Study EPO-ANE-3010 is a randomized, open-label, multicenter, international study to further examine the safety of the epoetin alfa used with standard supportive care (ie, packed RBC transfusions) compared with standard supportive care alone, when used to treat anemia associated with chemotherapy. This study is being done in subjects with metastatic breast cancer who are being treated or who will be treated with first-line chemotherapy with standard dose schedules of taxane monotherapy, or a taxane plus trastuzumab, or an anthracycline plus either a taxane or cyclophosphamide. The study hypothesis is that epoetin alfa, when used as supportive anemia care, does not increase the risk of tumor progression or death. The study treatment will be compared to the control treatment by comparing progression-free survival, ie, the time from the date a patient is randomized into the study to the date of the first documented disease progression or death. In addition to their chemotherapy, half of the subjects will be assigned to receive study drug (epoetin alfa) and half of the subjects will be assigned to standard supportive care for anemia.

Subjects treated with the study drug will receive standard supportive care (packed RBC transfusions) plus 40,000 IU epoetin alfa given subcutaneously once a week until 4 weeks after the last cycle of chemotherapy or until disease progression, whichever comes first.

Despite extensive efforts to facilitate recruitment, it has become evident that this study will not accrue sufficient patients in a timely manner to answer this important question. A number of challenges have been identified by J&JPRD and provide valuable insights into the feasibility of not only this study but also considerations when facing other similar studies. The lack of access to a suitable patient population leading to opening sites in international countries has led to challenges of facing unfamiliarity with the use of ESAs for the treatment of CIA or the lack of experience with the chemotherapy regimens specified in the protocol and current use of those regimens in a majority of their patients with breast cancer receiving treatment. Finally the lack of availability of quality imaging equipment and the ability to comply with the requirements for submission of radiographic data for centralized review has proven challenging.

7.4 Post-Marketing Surveillance

In compliance with current global regulatory policies, Amgen's and J&JPRD's pharmacovigilance units continually and systematically collect adverse events from multiple sources to conduct real-time and periodic medical assessments of single and aggregate cases to identify potential safety signals. Early detection of safety signals enables both companies to proactively develop and implement appropriate and timely risk management strategies. Both companies continue to closely monitor, assess and evaluate post-marketing surveillance reports for darbepoetin alfa and epoetin alfa.

The combined cumulative patient exposure for Aranesp, Procrit, and Eprex for all marketed indications since first marketing approval is 6 million person-years (1.6 million person-years for Aranesp, 1.6 million person-years for Procrit and 2.8 person-years for Eprex), of which the estimated cumulative exposure for the oncology indication is 1.5 million person-years (507,000 person-years for Aranesp; 790,088 person-years for Procrit; and 205,545 person-years for Eprex).

This represents substantial patient experience to date, in which safety is continuously monitored through spontaneous case reports of adverse reactions. Although post-marketing surveillance is an imprecise tool for detecting subtle safety signals, Amgen's

and J&JPRD's ongoing post-marketing surveillance programs have not identified any new or significant safety signals and has not been able to support or demonstrate any causal relationship of an adverse effect of Aranesp, Procrit or Eprex on tumor response, disease progression, or survival. TVEs are listed events across all approved indications in the product label. Overall, the frequency of the reports and observed reporting rate has remained stable, although the sensitivity of the reporting rate to changes and inaccuracies in the estimates of exposure must be emphasized. The frequency, nature, and severity of these reports are consistent with Amgen's and J&JPRD's prior experience and are adequately reflected in product labeling.

7.5 Re-evaluation of Ongoing Risk Management Plan and Need for Further Actions

Amgen and J&JPRD continue to evaluate the ongoing pharmacovigilance studies for darbepoetin alfa and epoetin alfa. Amgen and J&JPRD will expeditiously communicate the data from these studies and the status of any DSMB recommendations to FDA and to the healthcare community. Amgen remains focused on continuing its collaboration with the various study groups to ensure that the currently planned post marketing commitment dates are met. It should also be noted that, as reflected in a formal post-marketing commitment, 2 meta-analyses are planned to increase the statistical power and sensitivity of Amgen's individual pharmacovigilance studies. The first analysis will combine all 5 studies, resulting in 80% power to detect a hazard ratio of 1.15 or greater. The second analysis will combine the 2 breast cancer studies (PREPARE and ARA-03), resulting in 80% power to detect a hazard ratio of 1.3 or greater.

Based on the currently available data presented in this document, the risks of ESAs in patients with cancer have been comprehensively characterized and are adequately addressed in the current product labels. New data from the studies noted above, as well as ongoing studies for other ESAs, will help inform further risk management activities going forward. Of particular note is Amgen-sponsored Study 20010145, the results of which will be available shortly. Results from Study 20010145 are considered important for informing the future direction of risk management for ESAs in this setting. Data from J&JPRD study EPO-ANE-3010 will also be informative, although feasibility issues are acknowledged.

The continued focus of the ongoing risk management plans for darbepoetin alfa and epoetin alfa is the timely communication of risk information to FDA and the healthcare community and the scientifically appropriate assessment of risk through clinical investigation and data review. To date, identified areas of risk (tumor progression, survival, and CV/TE events) have been addressed through changes to the prescribing information, which were communicated to the healthcare community through a joint Dear Health Care Professional letter with Ortho Biotech. The current product labels for darbepoetin alfa and epoetin alfa clearly represent the established risks based on the accumulated product-specific safety data as well as observations from other products within the same therapeutic class. Amgen and J&JPRD will continue to assess all currently available and future data to further evaluate parameters that may be important for ensuring the adequacy of the communication of safety information in the label and/or providing insight into parameters currently hypothesized to be associated with outcomes.

J&JPRD's experiences in the conduct of EPO-ANE-3010 have highlighted the challenges associated with clinical investigations in this setting, and would need to be considered in any future plans.

8. Conclusions

ESAs are currently approved by worldwide regulatory agencies for the treatment of anemia due to concurrent chemotherapy in patients with non-myeloid malignancy (CIA). These approvals were obtained following the conduct and submission of robust randomized, placebo-controlled studies demonstrating significant reductions in the clinical need to administer transfusions.

ESAs are not labeled for treatment of anemia in patients receiving radiotherapy alone or in patients receiving neither chemo- nor radiotherapy.

Two recent reports have raised concerns about the safety of ESAs in unlabeled oncology patient populations:

- Amgen-sponsored Study 20010103, a randomized, placebo-controlled study in anemic patients with active cancer receiving neither chemo- nor radiotherapy demonstrated worse overall survival in the darbepoetin alfa group.
- The DAHANCA 10 study was an open-label randomized study in patients receiving primary radiotherapy for squamous head and neck cancer. Preliminary interim data identified an approximate 10% increase in disease progression in the darbepoetin alfa-treated patients. Definitive data from this study are awaited.

Both studies were conducted in patient populations not indicated in the prescribing information. These findings have been appropriately communicated, and are incorporated in the product label for all ESAs in the form of a boxed warning. Thus the current labeling for ESAs adequately reflects the identified potential risks of ESAs in these non-indicated populations. In response to the data from these recent clinical studies and in preparation for the 2007 ODAC meeting, Amgen and J&JPRD have performed a comprehensive review and analysis of the safety data (preclinical and clinical) for darbepoetin alfa, epoetin alfa, and other ESAs. This review represents the totality of evidence available to either company or in the public domain on ESAs that has been obtained to date. The focus of this review was to inform the risk/benefit profile of the use of these products in the currently approved CIA indication and to evaluate the adequacy of the currently approved labeling as well as ongoing pharmacovigilance programs. The findings resulting from this analysis are as follows:

(1) Preclinical data are reassuring with regard to the effect of ESAs on tumor progression and overall survival.

(2) Clinical data continue to indicate that ESAs are associated with an increased risk of venous thromboembolism. This risk has been accurately quantified and is reflected in the product labels.

(3) Comprehensive analyses of patient-level data from controlled clinical studies with darbepoetin alfa and epoetin alfa, when used in the setting of CIA, show a neutral effect on overall survival and tumor progression while demonstrating clear benefit in terms of reducing the need for blood transfusion.

(4) Updated meta-analyses of all ESAs involving over 8500 patients in 35 studies also demonstrate that the effect of ESAs on survival is neutral in patients with CIA (HR: 1.033, 95% CI: 0.922, 1.158).

(5) To date, four studies have been reported that show a significant, adverse effect on overall survival with ESA use in cancer: Epo-Can-20, BEST, ENHANCE and the 20010103 study of patients with active cancer not receiving chemo- or radiation therapy. The DAHANCA 10 study was stopped due to futility; definitive data from this study are awaited. All of these address experimental, unapproved indications.

(6) Only the 20010103 study and DAHANCA 10 are new since the 2004 ODAC meeting. In the same interval, four other new studies have shown neutral effects on survival: the 20040232 placebo controlled study in CIA across tumor types, the BRAVE controlled study in CIA in breast cancer, interim data from GELA study in CIA in NHL, and the Möbus controlled study in CIA in breast cancer.

(7) In CIA, the data presented at the 2004 ODAC concerning tumor progression and survival have become more extensive and robust. ESA administration does not appear to increase these risks in patients within this approved indication.

(8) Subject characteristic analyses cannot yet identify patients at special risk for adverse effects from ESA therapy. It is of interest that an achieved hemoglobin response predicts a favorable outcome, although this may represent simple confounding by patient status.

(9) The weight of evidence suggests that ESAs should not be used outside of the experimental setting to treat anemia associated only with active malignancy in

patients who have exhausted other options, or as a strategy aimed at hyperoxic radiosensitization.

(10) The existing and substantial weight of evidence presented here supports the continued appropriate use of ESAs in CIA as per the prescribing information. Ongoing pharmacovigilance studies will further inform the risk/benefit assessment in the near future.

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Appendix 1. Aranesp® USPI and Procrit® USPI

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Appendix 2. INFLUENCE OF ERYTHROPOIETIC STIMULATING AGENTS (ESAS)ON TUMOR PROGRESSION AND SURVIVA

INFLUENCE OF ERYTHROPOIETIC STIMULATING AGENTS (ESAS) ON TUMOR PROGRESSION AND SURVIVAL

LIST OF ABBREVIATIONS

Abbreviation	Definition
^{125}I-rHuEpo	Radiolabelled rHuEpo
ACTB	β-actin
AG490	Proposed inhibitor of Jak2
AKT	Protein kinase B
ALL	Acute lymphoblastic leukemia
AML	Acute myeloblastic leukemia
BACEC	Bovine capillary endothelial cell
bcr-abl	Philadelphia chromosome/translocation
BID	Twice per day
DA	Darbepoetin alfa
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme Linked Immunosorbent Assay
ELK	Transcription factor, target of an ERK
EMP-1	Erythropoietin mimetic peptide-1 (agonist)
EMP-9	Erythropoietin mimetic peptide-9 (antagonist)
EPC	Endothelial progenitor cell
Epo	Erythropoietin
EpoR	Erythropoietin receptor
ERK	Extracellular-regulated kinase
ESA	Erythropoiesis-stimulating agent
F-EpoR	Full-length Erythropoietin receptor
FlagEpoR	Flag/marker-tagged Erythropoietin receptor

Abbreviation	Definition
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GUSB	β-glucuronidase
HER2	Human epidermal growth factor receptor 2
hHSP90	Human heat shock protein 90
hHSP90a or c	Human heat shock protein a or c
HIF-1α	Hypoxia inducible factor-1 alpha
HSP70	Heat shock protein 70
HUVEC	Human umbilical vein endothelial cell
IC50	Concentration to achieve 50% inhibition
IGF-2	Insulin growth factor-2
IHC	Immunohistochemistry
IL-2	Interleukin-2
IL-7	Interleukin-7
ISCC	Invasive squamous cell carcinoma
Jak2	Janus kinase-2
Jak3	Janus kinase-3
kDa	Kilodalton
MAP(K)	Mitogen-activated protein (kinase)
MEK	MAPK/ERK kinase
MM	Multiple myeloma
mRNA	Messenger RNA (Ribonucleic Acid)
NF-κB	Nuclear factor - kappaB
NSCLC	Non-small cell lung cancer
PI3K	Phosphoinositol 3'-kinase
PlGF	Placental Growth Factor

Abbreviation	Definition
pO_2	Oxygen gas tension
PT	Photodynamic therapy
Q2W	Every two weeks
QD	Once daily
QW	Once per week
Raf	Gene coding for a protein kinase
RBEC	Rat brain capillary endothelial cells
RCC	Renal carcinoma cell
rHuEpo	Recombinant Human Erythropoietin
RT	Radiotherapy
RT-PCR	Reverse transcription-polymerase chain reaction
SCID	Severe Combined Immune Deficiency Syndrome
S-EpoR	Soluble Erythropoietin receptor
SHP-1	Src homology region 2 domain-containing phosphatase 1
ShRNA	Short hairpin RNA
SOCS	Suppressor of cytokine signaling protein
STAT-3 or 5	Signal transducer and activator of transcription-3 or 5
T-EpoR	Truncated Erythropoietin receptor
TIW	Three times per week
U/mL	Units per milliliter
VEGF	Vascular endothelial growth factor

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EXECUTIVE SUMMARY

In this paper the hypothesis that erythropoietin (Epo) and erythropoiesis-stimulating agents (ESAs) promote tumor growth has been broadly examined.

There are 3 key elements that underlie this hypothesis: (i) that Epo/EpoR directly stimulates/increases proliferation of cancer cells; (ii) that Epo/EpoR promotes tumor vascularization; and (iii) that Epo promotes tumor oxygenation with adverse clinical sequelae. The purpose of this review is to scientifically critique those papers that purport to support these 3 elements in an attempt to determine the level of scientific evidence supporting each of these ideas.

(i) Epo/EpoR does not directly stimulate nor increases proliferation of cancer cells

While erythropoietin receptor (EpoR) mRNA has been detected in tumors and EpoR protein is reportedly expressed in human tumors, an independent review of the literature (Osterberg et al., 2007) has reached similar conclusions to those presented below:

1. EpoR is not an oncogene. The EpoR gene is not significantly amplified or overexpressed in solid tumors and overexpression of constitutively activated mutant forms of EpoR does not transform cells.
2. EpoR hyperactivating mutations (EpoR truncations) result in erythrocytosis and are not a feature of malignancy. Similarly, conditions in which Epo is overexpressed (eg, Chuvash polycythemia), polycythemia results but with no increase in tumor incidence.
3. The EpoR gene is transcribed in most tissues and cell lines at low to moderate levels. Levels of EpoR mRNA are rarely elevated in tumors and cell lines above that seen in the normal tissue of tumor origin.
4. All commercially available anti-EpoR antibodies are non-specific and are unsuitable for immunohistochemistry. The most commonly used EpoR polyclonal antibody (Santa Cruz C-20) detects heat shock protein HSP70, not EpoR, in tumor samples.
5. EpoR mRNA levels do not necessarily correlate with fully functional EpoR protein levels due to methods that do not distinguish between alternatively spliced forms of the mRNA that encode proteins with attenuated or antagonistic functions.
6. EpoR protein synthesis does not necessarily correlate with cell surface expression or signaling of the EpoR. Less than 1% of EpoR normally gets to the surface of the cell due to inefficient processing, protein degradation, requirements for limiting accessory molecules for trafficking to the surface (eg, Jak2), requirements for limiting accessory molecules for intracellular signaling, and because of its short cell-surface half life.
7. Most studies that investigate the direct role of Epo:EpoR in signaling, proliferation, migration and survival of cancer cells are contradictory and not compelling. The majority of in vitro studies that report an effect have used suprapharmacological levels of rHuEpo (>10 U/ml), levels that are

unattainable in patients. They have used agents that are non-specific (eg, AG490 an EGFR, guanylyl cyclase C, bcr-abl, Jak3 and Jak2 inhibitor), have not used appropriate controls, and/or report modest (2-3 fold) effects on proliferation that are similar to background experimental noise.

8. All rodent tumor models (23 independent studies) have demonstrated that ESAs do not enhance tumor growth. Rather, to the contrary ESAs have been shown to increase sensitivity of tumor cells to radiation or chemotherapy.

(ii) Epo/EpoR does not promote tumor vascularization

1. ESAs do not mediate any consistent adverse effect on tumor angiogenesis in rodent tumor models.
2. The data do not support meaningful effect of ESAs on mobilization of endothelial progenitor cells.
3. There is no compelling data that circulating progenitor cells play a meaningful role in tumor vascularization in either preclinical models no in patients.

(iii) Improved tumor oxygenation does not adversely impact clinical outcomes .

1. It is well established that lower doses of ionizing radiation are required for tumor ablation if the oxygen tension within tumors is high. Indeed, improved responses to radiotherapy or chemotherapy in pre-clinical tumor models are associated with higher hemoglobin levels induced by recombinant erythropoietin and darbepoetin alfa.
2. Increased tumor oxygenation reduces hypoxia-regulated vascular endothelial growth factor levels and consequently tumor angiogenesis.

Taken together, these lead to the conclusion that there is no compelling scientific evidence for the expression or function of EpoR on cells from solid tumors or that ESAs mediate enhanced tumor vascularization. There is, though, compelling data that improved tumor oxygenation is associated with improved clinical outcomes

1.0 Introduction

Anemia occurs in a variety of disease states and has been treated successfully with the recombinant erythropoiesis-stimulating agents (ESAs) epoetin alfa, epoetin beta, and darbepoetin alfa. However, some investigators have suggested ESAs may promote tumor growth in cancer patients undergoing radiotherapy or chemotherapy, or in the absence of therapy. Three hypotheses have been proposed to explain how ESAs could potentially enhance tumor progression or antagonize tumor ablative therapy.

First, rHuEpo may directly promote tumor growth via an interaction with Epo receptors (EpoR) expressed on the surface of tumor cells. Putative EpoR expression is reported to be detected by several methods in a variety of human tumors and tumor cell lines. However, serious problems with EpoR-detection methodologies, conflicting data from different groups, lack of direct correlation between expression of EpoR protein and presence on the surface of tumor cells, and the lack of tumor promoting activity by ESAs in animal tumor models, bring these observations into question. It is theoretically possible that Epo:EpoR interactions could enhance tumor proliferation and/or reduce tumor cell apoptosis and thereby enhance tumor cell survival. However, the data surrounding the effect of rHuEpo on the proliferation and survival of cell lines in vitro are contradictory and not compelling, and no tumor promoting effect of exogenously administered ESAs in animal tumor models has been observed in 23 published studies in the presence or absence of tumor ablative therapy. This issue is a major focus of this report (Sections 4-6).

Second, ESAs may promote tumor vascularization, thereby promoting tumor growth. Angiogenesis, the process by which new blood vessels form from existing vessels, is the key mechanism by which tumors increase blood supply during early tumorigenesis or in response to hypoxia (Kerbel and Folkman, 2002). Emerging evidence suggests that vasculogenesis, the process by which new blood vessels form from endothelial progenitor cells during development, also may contribute to new blood vessel formation in adults. It has been hypothesized that ESAs might promote tumor angiogenesis through direct endothelial cell stimulation or promotes tumor growth by increasing tumor blood vessels through endothelial progenitor cell-mediated vasculogenesis. ESAs do not mediate any consistent effect on tumor angiogenesis in preclinical tumor models. Although rHuEpo does appear to cause modest mobilization of endothelial progenitor cells in some preclinical models, it is uncertain whether these cells contribute to the

tumor vasculature either in preclinical models or in the clinical situation. This issue is addressed in Section 7.

Finally, the pharmacodynamic effect of increased hemoglobin concentrations is known to improve oxygen delivery to cancer tissues. Recently it has been speculated that this may therefore be disadvantageous, and enhance tumor cell growth or angiogenesis. This is unlikely to be relevant for several reasons. First, improved tumor responses to radiotherapy and chemotherapy in rodent tumor models were associated with higher hemoglobin concentrations induced by treatment with recombinant human erythropoietin (rHuEpo) and darbepoetin alfa. Second, increased tumor oxygenation also decreases tumor vascular endothelial growth factor (VEGF) levels, resulting in a reduction of VEGF-pathway-regulated tumor neovascularization. Third, the beneficial effect of oxygen in enhancing tumor responses to ionizing radiation has been extremely well studied and documented, and exploited for improved clinical outcomes. Fourth, for decades numerous attempts of mimicking the beneficial effects of oxygen using small molecules as “hypoxic cell sensitizers” have been investigated. Finally, numerous attempts have been made, or are underway, to antagonize the molecules that are triggered by tumor hypoxia as legitimate anti-cancer therapeutics. Although superficially attractive, the hypothesis that tumor oxygenation may be disadvantageous, is not credible given the decades of clinical experience to the contrary. Because of this extensive preclinical and clinical experience, this hypothesis is only briefly addressed in this report (Section 8).

This paper reviews the current literature regarding the well-defined role of EpoR signaling and expression in hematopoietic cells and its uncertain role in tumor cell lines and tissues. The controversial role of ESAs and EpoR expression on endothelial cells and on tumor cell proliferation and survival in vitro and on endothelial progenitor cell mobilization and tumor progression in vivo is examined. Of note, an independent review of the literature (Osterborg et al, 2007) has provided similar conclusions as to these presented here.

2.0 Materials and Methods

Multiple broad searches of the biologic and medical literature were done on the Ovid system between 15 February and 15 March 2004 and between 19 February and 2 March 2007 using the following databases: EMBASE (1980-present), Ovid Medline (1966-present), BIOSIS Previews (1969-present), and Ovid MEDLINE in-process and

other non-indexed citations. Search strategies incorporated the concepts and terms appropriate to the research question. No restrictions were applied on language. The titles, abstracts, or both were read for all citations, and if the article was relevant, a full copy was obtained. Additionally, the reference sections of these papers were read and relevant papers not appearing on the original searches were identified. All publications determined to be relevant from inclusion in a critical, balanced, and thorough presentation of the subject matter are cited in this paper. Many of the citations described the effects of ESAs related to receptor expression and signaling, tumor cell proliferation, tumor growth, tumor progression, hypoxia, or angiogenesis.

3.0 Biology of EpoR in Erythroid Cells

3.1 Multiple EpoR Isoforms

EpoR protein is a type-1, single transmembrane receptor that is reportedly synthesized as several forms in erythropoietic progenitor cells including, full-length (F-EpoR), cytoplasmic truncated (T-EpoR), and soluble (S-EpoR). T-EpoR and S-EpoR contain the extracellular Epo-binding domain, but the cytoplasmic or transmembrane domains are truncated due to alternative splicing of transcripts (Nakamura et al, 1992). Knock-in and transgenic mouse studies have shown that under normal conditions T-EpoR can promote erythropoiesis when stimulated by endogenous mEpo and high levels of rHuEpo, but had compromised function to sustain erythroid progenitor survival and proliferation at low concentrations of rHuEpo and during stress-induced erythropoiesis (Li et al, 2003; Zang et al, 2001; Nakamura et al, 1998). T-EpoR is also reportedly expressed at higher levels on the cell surface though the mechanism for this is unknown (Motohashi et al., 2001). S-EpoR has been reported to act as an antagonist in neuronal tissues by competing with F-EpoR for binding to Epo (Sakanaka et al, 1998). However the physiologic role for this EpoR variant has not been established. It is not known if modulation of the 3 forms of the receptor at various points in the differentiation pathway plays specific roles during erythropoiesis.

3.2 Inefficient Cell Surface Expression of EpoR

Translocation of EpoR to the cell surface is an inefficient process with less than 1% of total cellular F-EpoR molecules found on the cell surface, a consequence of many factors including: the short half-life of EpoR (1 to 2 hours vs.12 hours for EGFR), inefficient processing for surface expression, and degradation within the endoplasmic reticulum, proteosomes and lysosomes (Suspino-Rosin et al, 1999; Kurten et al, 1996;

Hilton et al, 1995; Neumann et al, 1993; Sawyer and Hankins, 1993; Walrafen et al, 2005). EpoR transcription and translation do not always lead to surface expression of EpoR protein. Migliaccio et al (1991) reported various rHuEpo-dependent and rHuEpo-independent subclones derived from the hematopoietic cell line 32D expressed EpoR mRNA and protein (~62 to 66 kDa) when whole cell lysates were examined, but only the rHuEpo-dependent clones expressed surface receptor as determined by ^{125}I-rHuEpo binding and immunoprecipitation of EpoR bound to ^{125}I-rHuEpo (~59 to 66 kDa). The 59kDa EpoR band was only detectable when sensitive assays were used to identify the forms of EpoR able to bind rHuEpo. This suggests that the 59 kDa EpoR protein was functionally able to bind rHuEpo in these cells, and present at very low, undetectable levels when whole cell lysates were examined. Janus kinase-2 (Jak2) is also a key player in cell surface expression of EpoR and is absolutely required for trafficking EpoR to the cell surface. Jak2 binds EpoR protein in the endoplasmic reticulum, induces correct protein folding, and promotes surface expression (Huang et al, 2001). Thus if Jak2 is limiting, enforced expression of EpoR mRNA and protein may not increase surface levels of EpoR. This likely explains why EpoR mRNA overexpression in a hematopoietic cell line (UT7) did not increase total surface EpoR levels, determined by ^{125}I-rHuEpo binding (Hermine et al, 1996). However, additional accessory factors are also required to traffic EpoR to the surface of the cell as 32D clones without surface EpoR (Migliaccio et al,1991) express both cytoplasmic EpoR protein and Jak2 (DaSilva et al, 1994; Palaszynski and Ihle, 1984; Silvennoinen et al, 1993).

3.3 EpoR Intracellular Signaling

Epo responsive cell lines and hematopoietic cells have been used to assess the signaling pathways induced by Epo. Most EpoR proteins exist as preformed homodimers (Livnah et al, 1999; Constantinescu et al., 2001a) and are activated by Epo. The binding of Epo induces a conformation change in EpoR and brings 2 receptor-associated Jak2 molecules into close proximity (Syed et al, 1998; Seubert et al., 2003). This in turn induces Jak2 auto and trans-phosphorylation including phosphorylation of 8 conserved tyrosine residues located within the cytoplasmic tail of EpoR, that serve as docking sites for signaling adaptor proteins (reviewed by Constantinescu et al., 1999; Wojchowski et al, 1999; Constantinescu et al., 2001b).

EpoR stimulation results in activation of signal transducer and activator of transcription-5 (STAT5), phosphoinositol 3'-kinase (PI3K), and the MAP kinase (MAPK) pathways. Epo activates STAT5 (isoforms A and B) localized within the cytosol (Maucadel &

Constantinescu, 2005) and are phosphorylated directly by Jak2 after STAT5 associates with phospho-tyrosine residues on EpoR. Phosphorylated STAT5 translocates to the nucleus where it up regulates the expression of anti-apoptotic genes in hematopoietic cells (Wojchowski et al, 1999). However, additional signaling pathways are required to enhance differentiation and survival of erythroid progenitors (Li et al, 2003). PI3K binds to phosphorylated EpoR or to adaptor insulin receptor substrate-2 (IRS-2) proteins to promote cell growth and survival (Verdier et al, 1997) and differentiation through phosphorylation of AKT (Ghaffari et al, 2006). Activation of PI3K can have different effects in different cell types e.g., erythroid vs. myeloid cells. Therefore, activation through PI3K pathway is not predictive of a certain cellular response (Lewis et al, 2004). Signaling through the MAP-kinase pathway results in phosphorylation of a series of intermediate molecules including ERK1/2 (Wojchowski et al, 1999) that induce the transcription of growth-related proteins. Thus, activation of the MAP kinase pathway is hypothesized to induce the proliferation of erythroid progenitors (Zhang et al, 2003).

A number of molecules have been implicated in the negative regulation of EpoR signaling including, Src homology region 2 domain-containing phosphatase 1 (SHP-1), adaptor protein Lnk (Tong et al, 2005), and suppressor of cytokine signaling proteins CIS, SOCS-1 and SOCS-3 (Minoo et al, 2004; Jegalian and Wu, 2002; Hortner et al, 2002). In addition β-Trcp has been implicated in the ubiquitination and subsequent degradation of EpoR by the proteasome after Epo sitimulation (Meyer et al., 2007). The absence of negative regulation of EpoR signaling is associated with familial polycythemia due to cytoplasmic truncations of EpoR that remove SHP-1 and other suppressor binding sites (Gonda and D'Andrea, 1997; Arcasoy et al, 2002; de la Chapelle et al, 1993; Meyer et al., 2007). Although these patients have constitutive, life long signaling of EpoR and have erythrocytosis as a result, they do not have any reported increased cancer risk.

4.0 Putative Epo Receptor Detection and Expression in Tumor Tissues and Cell Lines

4.1 Detection of EpoR mRNA and Protein

The majority of studies that report data for EpoR protein expression are substantially compromised by inadequacies in the methodologies that have been employed. This is less of a problem with studies that examine EpoR mRNA expression with the caveat that mRNA expression does not necessarily equate to expression of a functional EpoR. For measurement of EpoR protein expression, there are no well-validated methodologies or reagents currently available. The approaches that have been most frequently used have significant limitations. Furthermore, measurement of EpoR mRNA or protein levels does not predict physiological responsiveness to endogenous Epo or to exogenous rHuEpo. Table 14 summarizes the limitations and advantages of the currently used methods to detect EpoR mRNA and protein.

Table 14. Limitations and Advantages of Currently Available Methodologies for Detecting EpoR on Tumor Tissue or Cells

	Limitations	Advantages
Immunoblotting (Western) using anti-EpoR antibodies	No ideal antibodies available (specific and sensitive) therefore multiple controls required Low level of EpoR expression and cross reactivity with non EpoR proteins obscures EpoR specific signals Cannot distinguish intracellular from membrane-bound or surface EpoR Does not differentiate functional from intracellular (inactive) receptors	Widely used technique Specific size band for EpoR can be identified (if specific antibody was available) Detects protein (intracellular and membrane) if specific antibody was available
IHC and flow cytometry using anti-EpoR antibodies	No suitable antibodies available (specific and sensitive) Low level of EpoR expression and cross reactivity with non EpoR proteins obscures EpoR specific signals. Does not differentiate functional from intracellular (inactive) receptors	Widely used techniques For IHC can use paraffin-embedded samples For IHC detects protein (intracellular and membrane) if specific antibody was available With flow cytometry can detect surface EpoR expression if validated conjugated antibody was available

Table 14. Limitations and Advantages of Currently Available Methodologies for Detecting EpoR on Tumor Tissue or Cells

	Limitations	Advantages
Flow Cytometry with conjugated Epo	Not a widely used technique and reagents not readily available	Can use conjugated Epo as reagent (more specific than antibodies)
	Typically used on liquid tumors or cell lines, very difficult to disassociate solid tumors	Detects surface EpoR
Radiolabelled Epo binding	Less widely available	Highly specific
	Requires fresh tissue and large numbers of cells	Detects dimerized receptor that is more likely to be functional and if intact cells are used can detect surface binding
	Cannot differentiate intracellular from surface binding in tissue sections	
RT-PCR	No correlation between EpoR mRNA levels and functional EpoR protein expression	Widely available technique
		Sensitive and specific for EpoR mRNA
	May not differentiate between active and inactive splice variants	
	Typically a qualitative measure of presence or absence of EpoR mRNA	
Laser capture of tumor cells with RT-PCR	No correlation between mRNA levels and functional EpoR protein expression	Sensitive and specific for EpoR mRNA
		Can use paraffin-embedded samples
	May not differentiate between active and inactive splice variants	Distinguish tumor cell and normal tissue expression
	Not widely available	
	Typically is a qualitative measure of presence or absence of EpoR mRNA	

The most widely used method for detecting EpoR mRNA is reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR is generally regarded as a very sensitive and specific method. However certain caveats exist with this method: 1) RT-PCR detects total EpoR mRNA, and does not differentiate between the active versus inactive splice variants as discussed above; 2) quantitative levels of mRNA are not typically assessed and thus direct comparisons of mRNA levels between cell types or tissues

cannot be made; and 3) detection of EpoR mRNA does not necessarily correlate with functional, cell surface expression of EpoR protein (Westphal et al, 2002; Abdalla et al, 2005; Migliaccio et al, 1991; Figure 4).

4.2 Specific and Sensitive anti-EpoR Antibodies Are not Currently Available

Immunoblotting (Western blots) and immunohistochemistry (IHC) are typically used to detect EpoR protein in transformed cell lines and ex vivo tumor tissue samples, respectively. Expression of EpoR is very low in normal erythroid progenitor cells, of the order of 200 – 1000 surface receptors (Broudy et al., 1991; Sawada et al., 1990; Sawyer et al., 1990), and tumor cells explaining why development of suitable antibody reagents is challenging. Similar to measurement of EpoR mRNA, detection of EpoR protein does not necessarily mean that cells or tissues express a functional EpoR. Investigators rely heavily on the use of commercially available anti-EpoR antibodies to detect EpoR protein by these methodologies. These antibodies have limited utility because they are all polyclonal antibodies with poor specificity to EpoR due to substantial cross-reactivity to non-EpoR proteins (Elliott et al, 2006a; Brown et al, 2007; Laugsch et al, 2006). The putative "anti-EpoR antibodies" used in most published studies come from several sources; the most commonly used are listed below:

Rabbit polyclonal antibodies to a human 20 amino acid C-terminal EpoR peptide (C-20) from Santa Cruz Biotechnology (detects 66 kDa putative EpoR protein);

Rabbit polyclonal antibodies to a human EpoR extracellular domain polypeptide (H-194) from Santa Cruz Biotechnology (detects ~52 or 66 kDa putative EpoR protein);

Rabbit polyclonal antibodies to a mouse 15 amino acid N-terminal EpoR peptide from Upstate Biotech (detects 72-78 kDa putative EpoR protein) (withdrawn 2005 due to non-specific interactions with proteins other than EpoR)

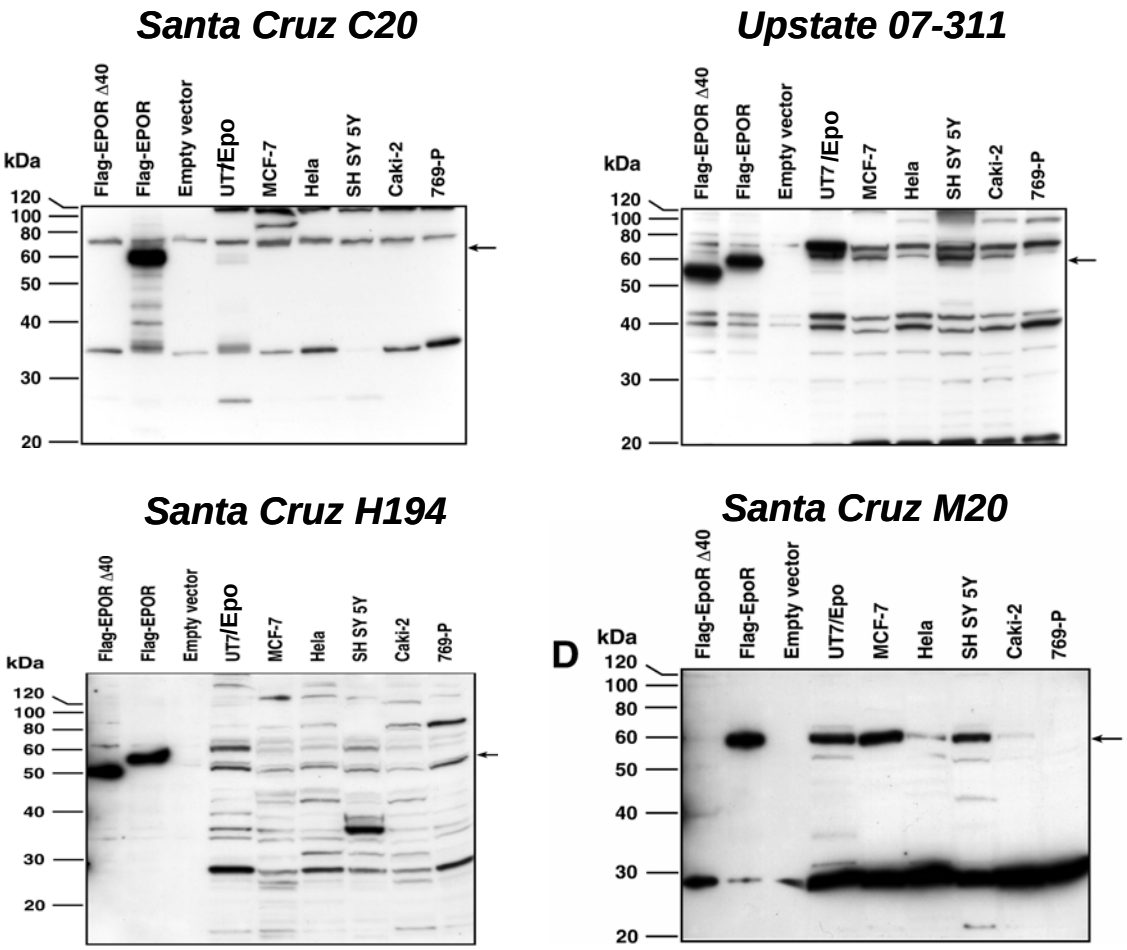
Sheep polyclonal antibodies to a human EpoR extracellular domain polypeptide from Calbiochem (detects 49 kDa putative EpoR protein)(no longer available)

mh2er16.5.1 monoclonal antibody from Genetics Institute (detects 66 kDa putative EpoR protein) (no longer available)

The peptides used to generate the antibodies are not specific to the EpoR protein. This results in polyclonal preparations that detect multiple proteins of a wide variety of sizes. The manufacturers did attempt to validate their antibodies; however, validation of antibody specificity is difficult and rarely performed adequately because of lack of appropriate positive and negative controls.

Investigators typically do not demonstrate antibody specificity and instead depend on validation from the commercial manufacturer or the laboratory that generated the antibody. The typical validation experiment conducted by either the manufacturer or the investigator assesses a loss of signal when the antibody is pre-incubated with excess antigen (typically the "EpoR peptide" used to generate the polyclonal anti-sera). This type of validation does not necessarily differentiate between specific antibody binding to EpoR and non-specific antibody cross-reactivity to other proteins (Elliott et al, 2006a; Brown et al, 2007). Typical Western blots with some of these antibodies are shown in Figure 32. In particular, C-20 polyclonal antibodies were shown to detect 4 proteins in tumor cell lines (35, 59, 66 and 100 kDa) while other "anti-EpoR" polyclonal antibodies detected over 20 proteins (Elliott et al, 2006a, Figure 32). This lack of specificity is not surprising given that all the antibodies are preparations of polyclonal anti-sera, they are used at relatively high concentrations, and exposures are extended in an attempt to detect the low amounts of EpoR in the samples.

Figure 32. Anti-EpoR Antibodies Detect Multiple non-EpoR Proteins on immunoblots and Thus Demonstrate Lack of Specificity for EpoR Protein



Polyclonal antibodies from commercial sources Santa Cruz C20, H-194 and M-20, and Upstate 07-311 were tested for EpoR specificity and demonstrated to be non-specific. All antibodies detected positive control FlagEpoR (~59 kDa – depicted by arrow to the right of blots) and 07-311 and H194 detected FlagEpoR Δ40 (~55 kDa) when transiently expressed in COS-7 cells. However, antibodies also detected multiple proteins different in size from recombinant EpoR in a panel of 6 human cell lines: UT7/Epo, megakaryoblastic leukemia; MCF-7, breast tumor; HeLa, cervical carcinoma; SHSY5Y, neuroblastoma; CAKI-2, kidney carcinoma; 769P, kidney carcinoma. Positive control cellular extracts containing recombinant FlagEpoR or FlagEpoRΔ40 (last 40 amino acids deleted) were tagged at the N-terminus with an 8 amino acid Flag sequence allowing verification of the position of the EpoR protein on the gel using anti-Flag antibodies (data not shown and Elliott et al., 2006a).

The confusion about the size of EpoR and misidentification of the EpoR band in immunoblot studies has resulted in misinformation about expression of EpoR protein on tumor cell lines and tumor tissues. The reported size of EpoR in immunoblot experiments varied according to which polyclonal antibodies were used and ranged from 49 to 78 kDa (Westenfelder et al, 1999; Benyo et al, 1999; Nagao et al, 1993;

Wesphal et al, 2002; Acs et al, 2003; Sawyer et al, 1993; Vogel et al, 2005a). However, the calculated mass of the Epo receptor, allowing for one carbohydrate chain, is considerably smaller at approximately 57 kDa (Elliott et al, 2006a) and the identity of EpoR in 59 kDa immunostaining bands has been confirmed by peptide sequencing (Laugsch et al, 2006; Elliott et al, 2006a; Elliott et al, 2006b). While most of the antibodies used to date detected EpoR protein when it is present at high levels, all of the anti-EpoR antibodies detected multiple proteins and the band corresponding to EpoR in immunoblot experiments was consistently misidentified (Elliott et al, 2006a; Brown et al, 2007; Laugsch et al, 2006). Some investigators reported detection of a correctly sized protein but assumed (incorrectly) this protein was not EpoR and reached their conclusions based on a misidentified protein (e.g., Migliacchio et al, 1991; Bao et al, 1999).

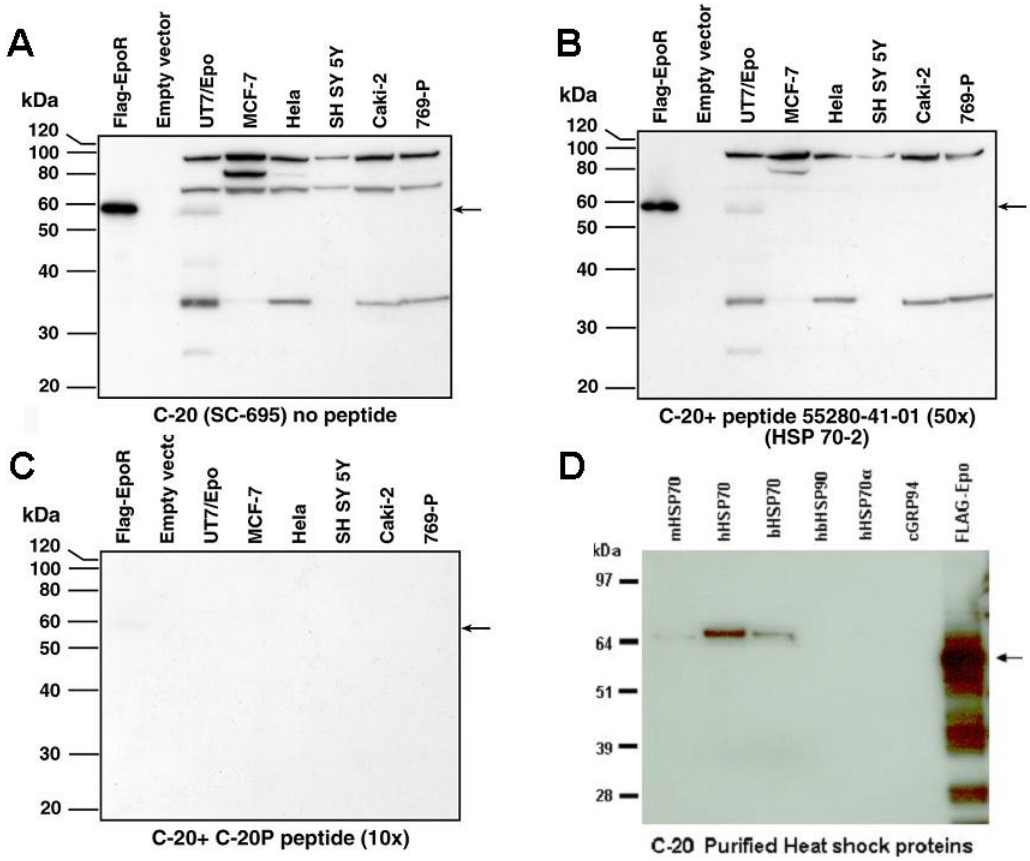
4.3 The Santa Cruz C-20 Antibodies Detect HSP70

The majority of published EpoR immunostaining and immunoblotting studies in tumor tissues or cell lines used the Santa Cruz C-20 polyclonal antibodies (e.g., as noted in Table 15; 20 of the 29 studies used C-20 or unspecified polyclonal rabbit antibodies from Santa Cruz Inc). The lack of specificity and misidentification of EpoR using the C-20 polyclonal antibodies is particularly notable because results based on this reagent are frequently cited as proof that tumors express a functional EpoR (e.g., Acs et al, 2001, 2002, 2003, 2004; Arcasoy et al, 2005a, 2005b; Eccles et al, 2003; Dagnon et al, 2005; Henke et al, 2006; McBroom et al, 2005; Mohyeldin et al, 2005; Pollio et al, 2005; Ribatti et al, 2003b; Winter et al, 2005). C-20, as noted above, reportedly detected a 66 kDa EpoR polypeptide, a protein substantially larger than EpoR. Investigators falsely assumed that the 66 kDa protein detected by C-20 was EpoR largely due to earlier reports that EpoR is 66 kDa in size and that staining was blocked when the antibody was preincubated with C-20 peptide, the immunizing peptide.

The 66 kDa protein is one of the most intensely staining proteins detected by C-20 and there was an unsubstantiated belief that the specificity of the antibody had been validated (Henke et al, 2006; Arcasoy et al, 2003). However, recent work (Laugsch et al, 2006; Elliott et al, 2006a, 2006b) showed that EpoR peptides were not identified in the 66-kDa band detected by C-20 and the intensity of this band did not decline following EpoR shRNA treatment, which abolishes EpoR mRNA and protein expression. In addition, the 66 kDa protein was detected by C-20 in 769P cells, a cell line that does not express EpoR mRNA (Figure 4). The 66 kDa immunostaining protein was recently

identified as HSP70 by peptide sequencing, strongly suggesting that many published studies using C-20 were detecting HSP70 in tumors and not expression of EpoR (Elliott et al, 2006a; Laugasch et al, 2006). Of particular note was the observation that C-20 polyclonal antibodies pre-absorbed with HSP70 peptides were able to specifically compete for binding to the 66-kDa band (Elliott et al, 2006a; Brown et al, 2007; Figure 33). These findings do not support the conclusion that the EpoR protein is present on cancer cells. Rather those authors have inadvertently confirmed the presence of HSP70 in tumor cells.

Figure 33. The 66 kDa Band Detected by Santa Cruz Polyclonal Antibody C-20 Was Identified to be HSP70



Peptide sequencing, antibody competition with HSP70 peptides and direct immunoblot detection identified the 66 kDa band detected by Santa Cruz polyclonal antibody C-20 as HSP70 not EpoR. A-C, immunoblots were prepared from the same cell lines described in Figure 32 and were incubated with C-20 polyclonal antibody as follows: A, C-20 antibody only (no-peptide); B, C-20 antibody preincubated for 1 hour with HSP 70-2 peptide (QQGRVEILAN DQGNRTTPSYVAFTDTER) mixed at antibody :peptide molar ratio of 1:50; C, C-20 antibody preincubated for 1 hour with C-20P peptide antigen (PYENSLIPAAEPLPPSYVACS) at antibody:peptide molar ratio of 1:10. B demonstrates that only the 66 kDa band is specifically competed with the HSP70-2 peptide, demonstrating that the protein at 66 kDa is HSP70. C demonstrates that competition with the peptide used to generate the antibody (C-20P) is an invalid control as all non-EpoR proteins are also competed. D, Immunoblot of purified heat shock proteins demonstrates the direct detection of murine (m), human (h) and bovine (b) HSP70 proteins at ~66 kDa in size with the C-20 antibody, but no detection of hHSP90, hHSP90α or canine (c) GRP94 proteins.(Elliott et al, 2006a; D, unpublished data)

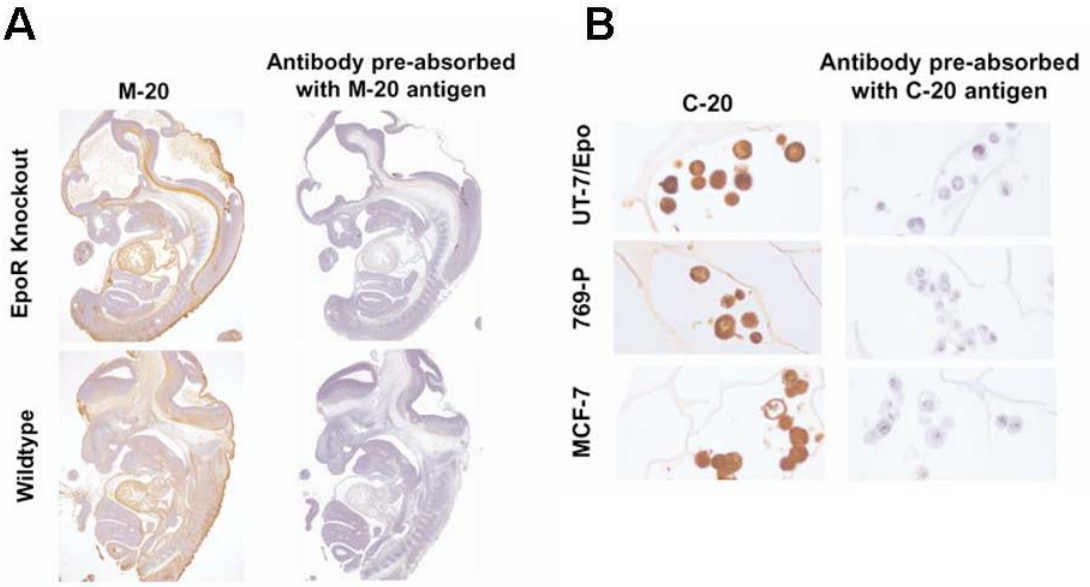
4.4 Existing anti-EpoR Antibodies Cannot Be Used in Immunohistochemistry Studies

Non-specific polyclonal antibody preparations are even less appropriate for IHC, where specific sized protein bands cannot be visualized to validate accurate binding of the antibodies to the antigen. Indeed, the Santa Cruz C-20 polyclonal antibodies preparation has been recently shown to be inappropriate for EpoR IHC studies (Brown, et al 2007; Elliott et al, 2006a).

C-20 strongly stained cells that had no or extremely low levels of EpoR mRNA (EpoR knockout embryo and renal tumor line 769P, respectively) equivalently to cells expressing high levels of EpoR mRNA (UT-7/Epo megakaryoblastic and wild-type embryo (Elliott et al, 2006a; Figure 34). Brown et al (2007) independently concluded that C-20 was not suitable for detecting EpoR using IHC according to the observation that the pattern of C-20 immunoreactivity on the tumor samples was significantly altered or abolished after preabsorption of C-20 to a HSP70 peptide (Brown et al, 2007).

Another preparation of polyclonal antibodies from Santa Cruz, (M-20 rabbit polyclonal antibodies raised to a 20 amino acid C-terminal EpoR peptide) showed some specificity to EpoR in immunoblot experiments (Elliott et al, 2006a; Laugsch et al, 2006; Figure 32). However, this antibody is also not suitable for IHC studies as revealed by similar staining of “EpoR” in wild type and EpoR knockout tissues (Figure 34; Elliott et al, 2006a). Yet, again this “anti-EpoR” antibody has been inappropriately used in tumor IHC studies to reportedly measure EpoR (Ceelen et al, 2007).

Figure 34. Santa Cruz Polyclonal anti-EpoR Antibodies M-20 and C-20 Are Not Suitable for Immunohistochemical Detection of EpoR



Santa Cruz anti-EpoR polyclonal antibodies M-20 and C-20 stain embryos and cell lines with no EpoR protein equivalently to embryos and cells with very high levels of EpoR protein. A, immunohistochemistry of EpoR knockout (top) and wild type (bottom) mouse embryos at 12.5 days of gestation with Santa Cruz anti-EpoR polyclonal antibody M-20 demonstrates equivalent staining. These data demonstrate that the M-20 antibody is not specific for EpoR and is staining non-EpoR proteins by IHC. Antibody competition with the peptide used as the antigen abolishes staining, demonstrating that this technique is not suitable as a control for antibody specificity. B, Anti-EpoR polyclonal C-20 (Santa Cruz) showed similar staining patterns of UT-7/Epo cells (top) that are dependent on Epo for survival and express high levels of EpoR mRNA (Figure 4A) and EpoR protein (Figure 5D); 769-P (middle) a renal carcinoma cell line with negligible levels of EpoR mRNA (Figure 4A) and undetectable EpoR protein (Figure 5D); and MCF-7, a breast carcinoma cell line with low levels of EpoR mRNA (Figure 4A) and high levels of EpoR protein (Figures 5D). These data demonstrate that Santa Cruz antibody detects non-EpoR proteins and thus provides false positives by IHC. Furthermore, preabsorption of C-20 antibody with a 5 fold excess of the C-20P antigenic peptide inhibited staining, demonstrating that this technique is not suitable as a control for antibody specificity. (Elliott et al., 2006a)

4.5 Correlation Between Detection of HSP70 by Santa Cruz C-20 Antibodies and Clinical Prognosis

Reports of over-expression of EpoR protein in tumors and a correlation between EpoR expression and prognosis (Henke et al, 2006) according to C-20 Western or IHC analyses is not actually detecting EpoR protein and instead is likely due to detection of heat shock proteins. HSP70 is present at elevated levels in tumors compared to normal tissues including cancer of the esophagus (Kawanishi et al, 1999), colorectal cancer (Kanazawa et al, 2003), pancreatic cancer (Lee et al, 1994), and bladder carcinoma, where it correlates with grade, stage and survival (Syrigos, et al 2003). Consistent with the observation of Henke (2006), cell surface over expression of HSP70 was also reported in head and neck cancer (Kleinjung et al, 2003). HSP70 has been associated

with poor clinical outcome (Vargas-Roig et al, 1998; Chang et al, 2003) and in increased metastatic potential (Kluger et al, 2005) in breast cancer. HSP70 is also a hypoxia inducible protein, explaining its elevation in hypoxic regions of tumors (Date et al, 2005; Weinstein et al, 2004; Gao et al, 2004; Patel et al, 1995) and is reported to be associated with increased proliferation (Vargas-Roig et al, 1997). HSP70 has been shown to have pro-growth, anti-apoptotic effects in gastric cancer cells (Zhao et al, 2005), where it shows the same staining pattern as the C-20 antibody (Isomoto et al, 2003).

Despite these serious methodological limitations with detecting EpoR protein, a number of studies have claimed to detect EpoR protein expression in tumor cell lines and tumor tissue. Studies that have evaluated EpoR protein expression on fresh tumor explants or paraffin-embedded samples from patients with cancer are summarized in Table 15. Again, the results must be interpreted with considerable caution since most of the studies used C-20 or other unvalidated, non-specific polyclonal antibodies to putatively detect EpoR. This has led to significant misinformation about the putative expression of EpoR in tumors. In reality, much of the published data in the field may be explained by cross-reactivity of the most widely used polyclonal antibody, C-20, to HSP70 protein. Although there are far fewer technical issues around measurement of EpoR mRNA it is important to once again highlight that expression of EpoR mRNA does not necessarily correlate with expression of a functional EpoR.

4.6 Detection of Cell-Surface EpoR Using rHuEpo-Binding Assays Has Generated Conflicting Results

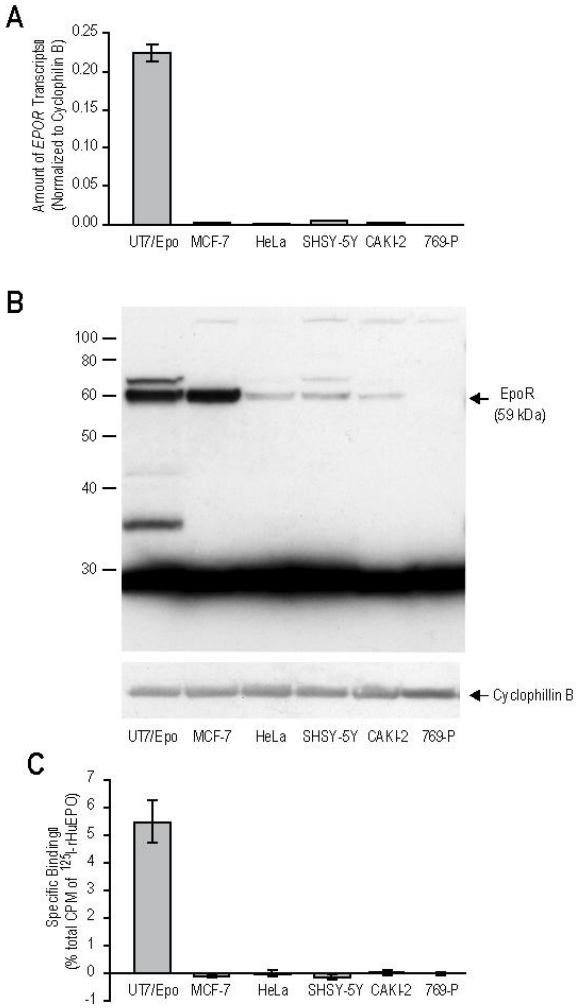
Since the available antibodies to EpoR are non-specific, rHuEpo-binding using ^{125}I-labeled or biotinylated rHuEpo provides an alternative methodology to detect surface EpoR. EpoR protein exists as a preformed homodimer on the surface of erythroid cells and binds a single Epo molecule at a high affinity and a low affinity site. The binding affinities of EpoR on primary erythroid progenitors range between 100 and 570 pM and receptor numbers between 135 and 1050 per cell (Broudy et al, 1991; Sawada et al, 1988). However, only a limited number of studies have used Epo-binding assays to investigate the presence of EpoR on the surface of tumor cell lines (Westenfelder and Baranowski, 2000; Masuda et al, 1993; Okuno et al, 1990; Um et al, 2007; LaMontagne et al, 2006). These studies have generated conflicting results that are difficult to reconcile. The studies that have investigated surface EpoR on non-hematopoietic and tumor cells have reported the binding affinity of EpoR was substantially (7- to160-fold)

lower than the binding affinity to primary erythroid progenitors and cell lines (Westenfelder and Baranowski, 2000; Masuda et al, 1993; Anagnostou et al, 1990; Broudy et al, 1988; Broudy et al, 1991). This is exemplified by a study using a rat pheochromocytoma which was reported to express EpoR with a binding affinity 160-fold lower than previously reported for EpoR on hematopoietic cells (Masuda et al, 1993). The significance of this unusually low binding affinity is unknown, but it does raise the possibility of technical challenges associated with this work and/or a non-functional receptor. It is difficult to imagine that an EpoR with low affinity characteristics of this type would be able to compete for access to Epo with high affinity, abundant EpoR on normal hematopoietic cells.

Other investigators have reported that although tumor cells may express both intracellular EpoR mRNA and protein, the EpoR receptor does not get to the surface at detectable levels (Abdalla et al, 2005; LaMontagne et al, 2006; Figure 35). In 2 breast cancer cell lines there was no detectable surface EpoR protein even though intracellular EpoR protein and mRNA was reportedly generated (LaMontagne et al, 2006). This is consistent with the data discussed in Section 3 that highlights the importance of Jak2 and other accessory molecules for cell surface expression of EpoR. Again consistent with these data, in hematological malignancies (B-cell lymphoma, mantle cell lymphoma and multiple myeloma) EpoR mRNA was detected but no Epo binding was observed (Abdalla et al, 2005). The presence of surface EpoR on 5 solid tumor lines using ^{125}I-rHuEpo binding studies has recently been examined. Full length EpoR protein was detected by immunoblot analysis (using Santa Cruz M-20 anti-EpoR antibodies) in tumor cell lines but no detectable specific Epo binding was observed (Figure 35). Thus, EpoR protein may be synthesized in some tumor cell lines but does not appear to necessarily migrate to the cell surface at detectable levels. A recent, well designed study demonstrated the difficulties involved in detecting cell-surface expression of the EpoR protein. An endocytosis assay was used to detect surface EpoR binding activity. This revealed that the differentiated neuroblastoma derived cell line SH-SY5Y had extremely low levels of surface EpoR (~20 receptors/cell; Um et al, 2007).

Thus, taken together, these studies demonstrate that the presence of detectable intracellular EpoR does not equate to EpoR trafficking to the surface of tumor cells. Even if EpoR does get to the surface at all, it is at extremely low levels and of uncertain biological significance.

Figure 35. EpoR Protein Is Not Trafficked to the Surface of Solid Tumor Cell Lines at Detectable Levels Even Though EpoR mRNA and EpoR Protein Is Expressed



Lack of correlation between levels of EpoR transcripts, levels of EpoR protein, and EpoR surface expression in tumor cell lines. Human cell lines were: UT7/Epo, megakaryoblastic leukemia; MCF-7, breast tumor; HeLa, cervical carcinoma ; SHSY-5Y, neuroblastoma; CAKI-2, kidney carcinoma; 769P, kidney carcinoma. Solid tumor cell lines were found to have undetectable levels of surface EpoR protein even though intracellular EpoR protein was synthesized. (A) Quantitative RT-PCR of EpoR mRNA in tumor cell lines. Similar data were obtained with 2 sets of primer/probes. (B) Western blot analysis of EpoR from total cell lysates using polyclonal M-20 anti-murine EpoR antibody. (C) Specific binding of ^{125}I-rHuEpo to cell lines. (unpublished data – S. Elliott, A. Sinclair)

In summary, EpoR mRNA expression has been detected in numerous normal tissues as well as in tumor cell lines and tumor tissues. However, the conflicting results and limitations of the reagents and methodologies in many experiments highlight the need for caution when interpreting the data. In addition many studies do not discriminate

between functional full-length EpoR and EpoR with attenuated or antagonistic properties. Although only limited studies have been done, surface EpoR is at extremely low or undetectable levels in tumor cells analyzed thus far, and if detected, the affinity of Epo:EpoR interaction may be reduced compared with hematopoietic cells. Taken together the data are consistent with the view that EpoR mRNA expression on normal nonhematopoietic and tumor cells is most likely due to the retention of very low, physiologic tissue expression of EpoR mRNA and is unlikely to be a driver of tumor progression**.**

5.0 EpoR Does Not Have the Properties of an Oncogene

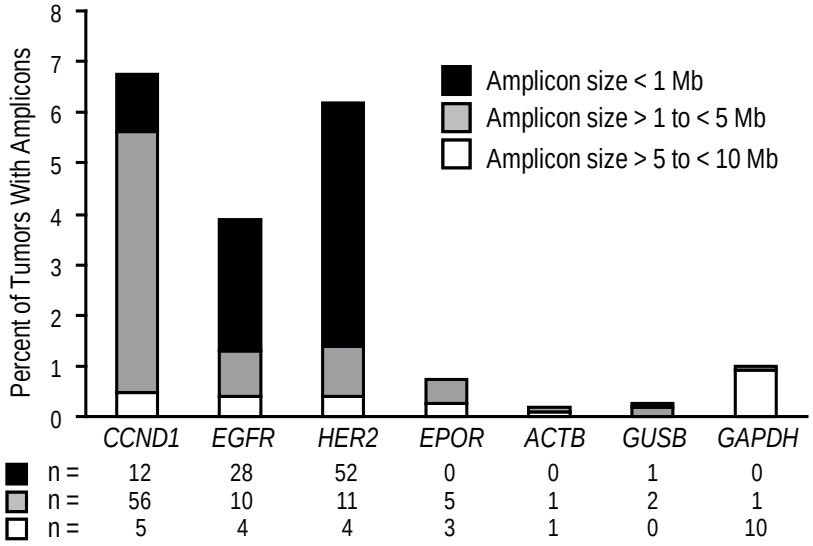
5.1 The EpoR Gene Is Not Amplified in Solid Tumors

A common phenomenon of tumor formation is the amplification of proto-oncogenes such as HER2 (Parkes et al, 1990), EGFR (Reissmann et al, 1999), CCND1 (Reissmann et al, 1999; Szepetowski et al, 1992), and c-MET (Rege-Cambrin et al, 1992) that provide a selective advantage for tumor cell growth and survival through protein over expression. Two erythroleukemic cell lines have been reported to have amplified genomic EpoR that correlates with elevated EpoR mRNA levels (Migliaccio et al, 1993; Ward et al, 1992; Chretien et al, 1994). This is not unexpected since it would be presumed that over expression through EpoR gene amplification would have a potential selective advantage in erythroleukemic cells.

The potential for the EpoR gene locus to be amplified in solid tumors has been investigated by genomic analysis of 1,084 tumors. This study identified that the locus was amplified in few (< 0.7%) tumors and only in large amplicons that encompasses many genes in addition to EpoR (> 1 Mb and < 10 Mb, Figure 5). When present, these large amplified regions were present at no more than 2 to 3 times the normal copy number. In contrast, amplified copies of EGFR, CCND1, and HER2 were detected frequently in small amplicons (< 1 Mb), and were amplified in 3.5% to 6.7% of tumors at up to > 9 times the normal copy number. The significance of the larger amplicons is that the EpoR gene is likely a “passenger” gene that is being co-amplified with a gene that may truly play a role in the oncogenic process, or that this represents a random process of DNA amplification that is characteristic of tumor cells. Similarly, the low copy number (2 to 3-fold) of EpoR amplification when observed supports this notion. Changes of this type were seen with other control, non-oncogenic loci such as β-Actin or GAPDH. Furthermore, overexpression of a constitutively active mutant of EpoR did not transform

fibroblasts (Longmore and Lodish, 1991). These data demonstrate that amplification of the EpoR gene itself, if it occurs at all, is a rare event in solid tumors and is not a primary driver of tumor formation and progression.

Figure 36. The *EpoR* Gene Was Not Amplified Above Other Non-Oncogenes in Solid Tumor Samples



The *EpoR* gene was not amplified in solid tumors above that seen with other non-oncogenes in an analysis of 1084 tumors from 15 different tumor types. Analysis was performed by quantitative genomic microarray analysis. Percent of tumors demonstrating genomic amplification of oncogenes cyclin D1 (*CCND1)*, *EGFR,* and *HER2*; non-oncogenes β-actin (*ACTB)*, β-glucuronidase *(GUSB),* and glyceraldehyde-3-phosphate dehydrogenase *(GAPDH);* and test locus *EpoR*. The numbers of tumors with amplicons are shown below the x axis. (unpublished data – P. Kassner, V. Watson, A. Sinclair , Amgen Inc.)

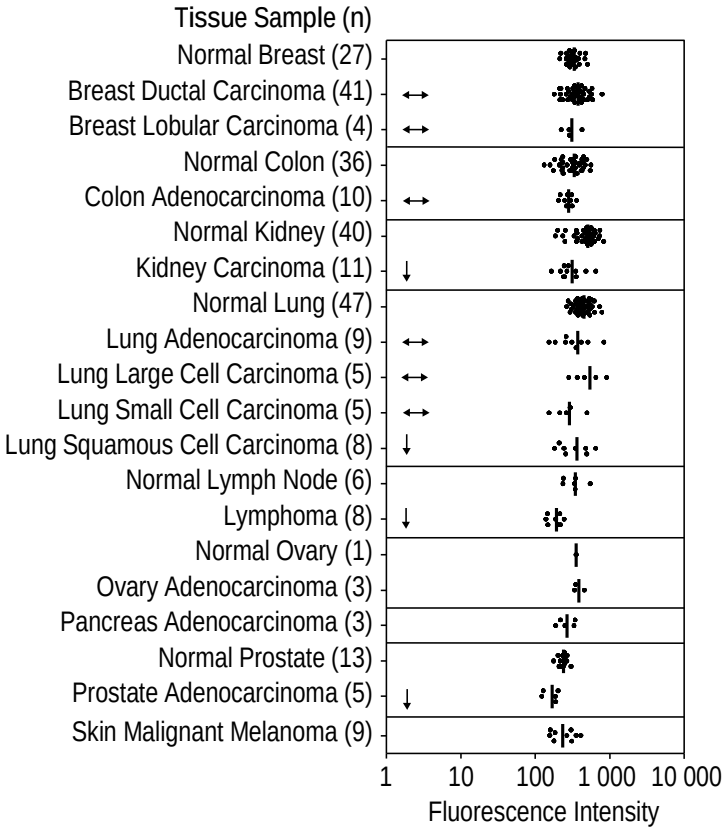
5.2 EpoR mRNA Is Not Overexpressed in Cancer vs. Normal Cells

A number of studies have used non-quantitative RT-PCR to demonstrate that EpoR mRNA was expressed in tumor cell lines and tumor tissues derived from breast, kidney, colon, stomach, pancreas, prostate, female reproductive organs, liver, lung, brain, thyroid, melanocytes, head and neck, and hematopoietic neoplasia (Acs et al, 2001; Aracsoy et al, 2002b; Yasuda et al, 2003; Westphal et al, 2002; Westerfelder and Baranowski, 2000; Arcasoy et al, 2005; Feldman et al, 2006; Yasuda et al, 2002; Yasuda et al, 2001; Dagnon et al, 2005; Batra et al, 2003; Selzer et al, 2000; Kumar et al, 2005; Lai et al, 2005; Mohyeldin et al, 2005; Gong et al 2006; Yates et al, 2006; Rossler et al 2004).

Westphal et al (2002) found that 20 out of 23 tumor cell lines expressed EpoR mRNA. They reported that the detection of mRNA correlated with immunostaining of EpoR protein in most cell lines using a currently unavailable monoclonal antibody raised to EpoR. Although the specificity and sensitivity of this antibody cannot be confirmed, it is worrying that in a urinary bladder carcinoma cell line and in immortalized keratinocytes, no EpoR mRNA was detected although these cells were reported to be positive for EpoR protein (Westphal et al, 2002). In addition there is concern about the lack of specificity of the anti-EpoR antibody as multiple bands were detected and the EpoR protein was incorrectly reported to be 66 kDa (as described above). Furthermore, the investigators found no effect on the growth of tumor cell lines in response to rHuEpo despite "expression" of EpoR protein (Westphal et al, 2002). Detection of EpoR mRNA without identification of EpoR cytoplasmic or cell surface protein was also recently reported by Abdalla et al (2005) using fresh tumor cells from B-cell malignancies. Thus, the detection of EpoR mRNA expression does not always equate to the presence of a functional Epo response, particularly in non-hematopoietic cells.

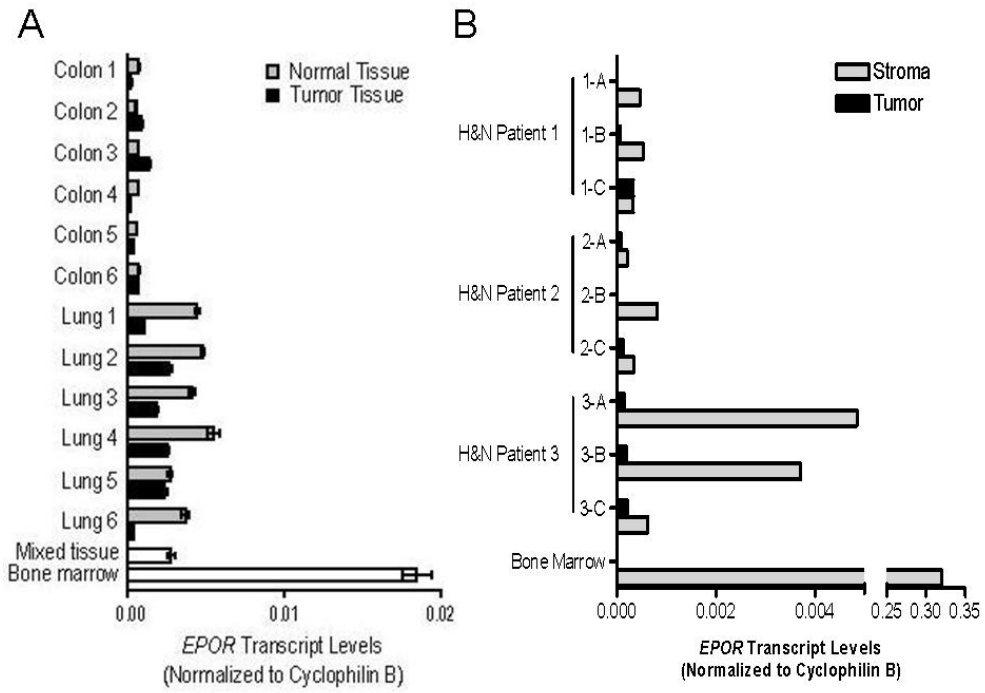
The EpoR gene is transcribed in many normal tissues, including kidney, heart, brain, endothelium, and smooth muscle (Hardee et al, 2006); however, there are no reports that tumors express levels of EpoR mRNA above those observed in normal tissues. Therefore, EpoR mRNA detection in tumors is most likely due to the vestigial expression from the tissue of tumor origin. In support of this notion, Feldman et al (2006) reported similar levels of EpoR mRNA in neoplastic prostate and normal prostate tissues using quantitative RT-PCR. Also, levels of EpoR mRNA in head and neck squamous cell carcinoma samples were similar to levels in paired normal samples (Winter et al, 2005). A comprehensive analysis of EpoR mRNA levels in breast, colon, lung, lymphomas, ovary, prostate, stomach, ileum, and kidney tumor tissues by microarray found EpoR mRNA levels were no higher than levels observed in normal counterpart tissues (Sinclair et al, 2005; Figure 6). Similar levels of EpoR mRNA were also found in matched tumor and non-tumor samples from lung, colon, or head and neck cancer patients (Sinclair et al, 2005; Figure 7). These data demonstrate that EpoR mRNA is present in most normal and tumor cells, and that levels of EpoR mRNA in tumors are not elevated above normal tissues.

Figure 37. EpoR mRNA Levels Were Not Elevated in Tumor vs. Normal Tissues in Multiple Different Tissue Types



EpoR mRNA levels in tumors were not elevated above levels seen in normal tissues of tumor origin. Comparative microarray analysis of 121 tumor and 170 normal tissues from breast, colon, kidney, lung, lymph node, ovary, pancreas. prostate, and skin samples. Closed circles represent mRNA levels from individual samples using EpoR probe 396_F_AT (EpoR exon 8). Other probe sets yielded similar intensity profiles (data not shown). Horizontal, double-headed arrows indicate no statistical difference in EpoR mRNA levels between normal and tumor tissues. A single-headed arrow indicates a significant ($p < 0.05$) reduction in levels of EpoR transcripts in tumor tissues compared with normal tissues. No statistical analyses were performed on pancreatic samples because of the lack of a normal control, or on ovary and melanoma samples because of their small sample sizes. (unpublished data – G. Arnold, A. Sinclair, Amgen Inc.)

Figure 38. Levels of EpoR mRNA Were Similar in Patient Matched Normal vs Tumor Samples From Colon, Lung, and Head and Neck



EpoR mRNA levels were not elevated in tumor samples relative to patient matched normal controls. Quantitative RT-PCR was used to determine levels of EpoR mRNA in normal and tumor tissues relative to levels of cyclophilin B mRNA. (A) Levels of EpoR mRNA in patient-matched normal vs colon and lung tumor samples. Mixed tissue (pooled RNAs from normal human tissues as a reference standard) and bone marrow samples were included as normal non-hematopoietic and hematopoietic controls. (B) Quantitative RT-PCR was used to determine levels of EpoR mRNA levels relative to levels of cyclophilin B mRNA in laser dissected normal stroma (epithelial, endothelial and smooth muscle cells) and tumor cells from 3 head and neck cancer patients. Numbers 1 to 3 refer to the patient ID number and A to C refers to different isolations from different regions from the same tumor. Normal human bone marrow was used as a positive control. (unpublished data – N. Rogers, I. Archibeque, A. Sinclair, Amgen Inc.).

The RT-PCR methodology used to detect EpoR mRNA in tumor cells does not typically distinguish between various alternatively spliced forms of the transcripts. High levels of alternatively spliced EpoR mRNA that would generate truncated and soluble EpoR proteins have been reported in breast, colon, lung, ovarian, and prostate tumors (Arcasoy et al, 2003). These findings are supported, in part, by the detection of soluble EpoR in conditioned media from a variety of tumor cell lines using an Epo binding ELISA

(Westphal et al, 2002). The physiological relevance of alternatively spliced EpoR transcript forms has not been determined in normal nor tumor cells. However, the EpoR isoforms may have attenuated (Li et al, 2003) or antagonistic (Sakanaka et al, 1998) function and may compete with full length EpoR for ligand binding.

6.0 In Vitro and in Vivo Functional Studies

6.1 Suprapharmacological Concentrations of ESAs Are Used in Many in vitro Studies

One important consideration to take into account when reviewing the in vitro EpoR functional data is the dose of ESA used in the study. Normal serum concentrations of Epo range from 0.005-0.025 Unit/mL in healthy, non-anemic individuals. Epo responsive cell lines (eg UT-7 Epo UT-7, TF-1, or HCD) and hematopoietic cells (eg, bone marrow) are typically very responsive to Epo in vitro with dose dependent increases in response in the 0.1 to 1 U/mL range and maximal stimulation occurring at approximately 1 U/mL with 10-fold increases in cell proliferation in 2 to 3 days (Rich 1994, Elliott et al 2005, Komatsu 1992, 1993; Sawafa 1987). It has been reported that single clinical doses of 150 to 2400 U/kg rHuEpo (administered either intravenously or subcutaneously) would result in maximum serum concentrations (C_{max}) of rHuEpo of approximately 3 to 10 U/mL (Ramakrishnan et al, 2004). The peak serum concentrations at steady state in patients receiving product label-recommended doses of rHuEpo (150 U/kg TIW or 40,000 U QW) range from 0.5 to 1.2 U/mL (Amgen data on file).

Most in vitro studies that assessed EpoR signaling, tumor cell proliferation and/or survival, or endothelial cell proliferation used rHuEpo doses that were several orders of magnitude above the physiologic concentration of endogenous Epo and also above the maximal levels obtained in patients after exogenous administration of rHuEpo. Typically, in vitro effects of ESA treatment were only observed at suprapharmacological concentrations of greater than 1 U/mL and many studies used concentrations in excess of 10 U/mL.

6.2 EpoR Signaling in Tumor Tissues and Cell Lines

Epo signaling pathways in tumors and transformed cell lines have been investigated in a number of studies and in some instances compared to survival, proliferative, and migration responses in vitro and in vivo. The interpretation of data proposing Epo:EpoR induced signaling and responses in tumor lines is confounded by the frequent use of

serum starvation, suprapharmacological doses of rHuEpo (> 10 U/mL), the lack of appropriate vehicle controls, responses that are not significantly above background and conflicting data on the relationship between Epo:EpoR signaling and a physiological response (eg, survival, proliferation, and migration).

Some in vitro studies have not defined or examined specific signaling pathways in tumor cells in response to rHuEpo. This is exemplified by the reported general increases in tyrosine phosphorylation in cell lines when exposed to suprapharmacological doses (250 U/mL rHuEpo in the absence of critical vehicle controls that could contain serum, insulin, IGF-1 or other proteins that could provide a false positive response) of rHuEpo which were suggested to be indicative of proliferative (Acs et al, 2001) and survival responses (Acs et al, 2003) under serum free conditions. Other studies have defined some signaling pathways. Modest increases in the translocation of NF-κB to the nucleus and up-regulation of survival gene transcription has been reported in Ewing's sarcoma cell lines when exposed to rHuEpo (30 to 100 U/mL in the absence of critical vehicle controls) but a physiological response such as inhibition of cell death was not examined (Batra et al, 2003). These data are contradicted by another study that reported 10 U/mL rHuEpo (with critical vehicle controls not apparently used) induced Jak2 phosphorylation and subsequently inhibited the NF-κB induced anti-apoptotic gene transcription to enhance the apoptotic effect of chemotherapeutic agents in renal carcinoma cell lines (Carvalho et al, 2005). None of these studies used cell lines in which EpoR protein was confirmed to be expressed using a validated methodology. Equally, given the suprapharmacological doses of rHuEpo employed, and the absence of critical controls, the observed effect could be solely due to contaminating proteins in the vehicle preparation.

Some studies have suggested that head and neck carcinoma cell lines express EpoR and signal in response to rHuEpo via Jak2 and STAT5 to enhance invasion and proliferation (Lai et al, 2005). Phosphorylated STAT5 levels were minimally elevated (2- to 3-fold) in serum starved cells at doses of rHuEpo (1 U/mL) that reportedly enhanced migration of head and neck carcinoma cell lines (2- to 3-fold). Proliferation was reported at a maximum of 2-fold above that observed with untreated cells at 100 U/mL rHuEpo (suprapharmacological) after 6 days (Lai et al, 2005). This study did not use critical vehicle controls which could again account for the modest responses observed. Another study reported that 10 to 100 U/mL rHuEpo may enhance survival of melanoma cell lines upon hypoxia or chemotherapeutic exposure via MAP kinase

pathway activation under serum free conditions (Kumar et al, 2005). Interestingly, only an increase in phosphorylation of Raf and MEK were observed and not downstream ERK1/2 nor ELK. Taken at face value, this would imply a block in signal transduction to downstream effector proteins may have occurred in these cell lines. Similarly, a very modest increase in AKT phosphorylation (less than 2-fold) in melanoma cell lines was seen upon exposure to high doses of rHuEpo up to 100 U/mL and was reported to modestly increase (20%) survival under hypoxic conditions (Kumar et al, 2006). The survival data were contradictory in this study as no increased survival was observed with a different viability assay, thus questioning the significance and reproducibility of the data (Kumar et al, 2006). Similar to the studies discussed above, it is unclear if vehicle controls were used in these studies.

A small increase (~2 fold) in STAT5 phosphorylation was reported to play a role in the modest proliferative response (2- to 3-fold) in 3 out of 5 prostate tumor lines (Feldman et al, 2006). Modest ERK1/2 activation (2-fold) has been reported in the MCF-7 breast tumor cell line upon exposure to rHuEpo and was suggested to correlate with a 2-fold increase in migration (Lester et al, 2005) with serum starvation. Similarly, approximately a 2-fold increase in phosphorylation of STAT5, AKT, ERK1/2 and NF-κB were reportedly stimulated in a differentiated neural cell line SH-SY5Y and was reported to correlate with a 40% to 60% reduction in apoptosis when exposed to staurosporine (Um et al, 2007).

6.2.1 EpoR Signaling Examined Using Non-Specific Jak2 Inhibitor, AG490

Several studies have suggested that inhibition of signaling via Jak2 antagonism with the small molecule AG490 implicates the Epo:EpoR axis in tumor cell responses (Mohyeldin et al, 2005; Lai et al, 2005; Belenkov et al, 2004; Carvalho et al, 2005). For example, Mohyeldin et al (2005) reported that high doses of AG490 (20 μM) inhibited rHuEpo induced (10 U/mL) Jak2 phosphorylation and invasion by ~60% in 2 head and neck cancer cell lines in vitro. Similar studies by Lai et al (2005) reported AG490 (1 μM) inhibited rHuEpo (1 U/mL) induced invasion in head and neck cancer cell lines under serum free conditions. However, the claim that AG490 is specific for Jak2 and EpoR signaling is erroneous. AG490 was first described as a potent EGF receptor (EGFR) antagonist with IC50 potencies of 100 nM on EGFR and 13.5 μM on Her2 autophosphorylation in enzyme assays (Gazit et al, 1991). Furthermore, EGF induced cell proliferation was reportedly inhibited with AG490 with an IC50 of 3.5 μM in the absence of serum and an IC50 of 25 μM in the presence of serum (Gazit et al, 1991).

Recent studies have reported that 100 nM of AG490 could inhibit EGF induced neurite outgrowth in neuronal cell line cultures (Goldshmit et al, 2004), though data was not shown. AG490 has also been reported to antagonize Jak3 in cell based assays such as inhibiting IL-2 induced T-cell proliferation with an IC_{50} of 10-25 μM and Jak3/STAT5 phosphorylation with an IC_{50} of~25 μM (Kirken et al, 1999). Another study reported similar results with AG490 inhibiting Jak3 in T-cell based proliferation and signaling assays with IC_{50} ~50 μM (Wang et al, 1999), AG490 also inhibited Jak3/STAT3 phosphorylation and viability of colon cancer cell lines with an IC50 of 40 to 80 μM (Lin et al, 2005) and also inhibited IL-7 induced Jak3 phosphorylation at 50 μM in primary human thymocytes (Sharfe et al, 1995). Furthermore, AG490 has also been reported to antagonize guanylyl cyclase C with an IC_{50} of 6.4 μM in enzyme assays (Jaleel et al, 2004) and bcr-abl at a potency of 29 μM in cell based assays (Kaur et al, 1994). Thus, studies that use AG490 to specifically antagonize Epo:EpoR induced cellular responses are not valid since AG490 is a broad inhibitor if kinases and inhibitory effects observed are most likely due to antagonism of other pathways such as EGF:EGFR. This is particularly relevant when examining cell lines from head and neck cancers that express high levels of EGFR and is the target for cancer therapy (reviewed by Reuter et al, 2007). Even if AG490 inhibits Jak2 to some degree, Jak2 is used by over twenty different receptor complexes so inhibitory effects can not be attributed to specific antagonism of Epo:EpoR signaling (Seidel et al, 2000).

6.2.2 Intracellular Signaling in Response to Suprapharmacological Doses of rHuEpo Does Not Correlate With a Cellular Response

The reported activation of signaling pathways via Epo:EpoR pathways are not always associated with a cellular response. Gewirtz et al (2006) examined breast cancer cell lines and an erythroleukemic cell line and found that although one breast tumor cell line (MCF-7) and the erythroleukemic cell line (HCD57) appeared to signal through Epo:EpoR, rHuEpo, even at doses of 10 U/mL, they did not stimulate cellular proliferation or inhibit cell death associated with chemotherapeutic drug exposure (Gewirtz et al, 2006). In another study, Hardee et al (2006) reported that suprapharmacological doses of rHuEpo (up to 100 U/mL) induced AKT and ERK1/2 phosphorylation in a rat mammary adenocarcinoma cell line and appeared to protect the cells from serum starvation and taxol-induced apoptosis in vitro. However, in vivo, though AKT phosphorylation was observed in tumors after a dose of 2,000 U/kg rHuEpo, no effect was observed on tumor growth or on inhibition of taxol induced tumor

regression (Hardee et al, 2006). These data suggest that even if rHuEpo induces signaling in solid tumors, it does not automatically correlate with a physiological effect in vivo.

Other studies support the findings that apparent signaling through Epo:EpoR may not consistently translate to cellular responses. A cervical carcinoma cell line (HeLa) was engineered to highly over express EpoR and was reported to increase STAT5 phosphorylation (~20 fold) and NF-κB translocation to the nucleus (~10 fold) upon exposure to suprapharmacological doses (25 U/mL) of rHuEpo (Pajonk et al, 2004). Although the authors found a modest increase in clonal growth with EpoR over expression (~25% increase in clonal growth) and high doses of rHuEpo exposure (25 U/mL), rHuEpo did not protect the cells from radiation induced death (Pajonk et al, 2004). In another study, investigators compared the signaling and growth responses of non-small cell lung carcinoma cell line (H838) with a megakaryoblastic cell line (UT7) in response to rHuEpo (Dunlop et al, 2006). While the investigators reported rHuEpo (10 U/mL) induced similar levels of STAT5, AKT and ERK1/2 phosphorylation in both H838 and UT7 cell lines (~5- to 20-fold increase), only UT7 cells demonstrated a proliferative response upon treatment with rHuEpo (Dunlop et al, 2006).

There are other examples of dissociation between intracellular signaling and cellular responses that are of concern. In vivo, Epo:EpoR interactions reportedly activated STAT5 in tumor xenografts, yet inexplicably there was no evidence for this effect in the same cell lines examined in vitro (Yasuda et al, 2003). In an attempt to address the specificity of response, those investigators injected S-EpoR and anti-Epo antibodies directly into tumors in ex-vivo organ cultures. They reported that Jak2 and STAT5 signaling were reduced because of inhibition of autocrine or paracrine Epo:EpoR signaling in tumors (Yasuda et al, 2002). Multiple sequential injections with large quantities of S-EpoR protein (0.3 to 1.6 μg/mg of tissue) and anti-Epo antibodies (32 μg/mg of tissue) were required for a non-dose related, inconsistent effect (0- to ~10-fold reduction in STAT5 phosphorylation). Because appropriate negative controls were not used in this study, and the anti-Epo antibody or S-EpoR were not validated for their antagonistic activities, one cannot rule out the possibility that the effect was due to nonspecific inhibition of tumor cell growth due to the direct injection of large amounts of material in general and not to anti-Epo activities of antibodies or soluble EpoR protein, per se. Since Jak2 and STAT5 are not only activated by Epo but also by numerous other growth factors (Seidel et al, 2000), the relevance of these data to Epo:EpoR

signaling are unclear. The most likely explanation is that non-Epo growth factors were active in vivo and/or contaminating stromal cells in vivo may have contributed to the phosphorylated STAT5 signal. Generally, experiments that analyze isolated signaling pathways in vivo or ex-vivo are difficult to interpret in terms of their physiologic or pathologic significance as they are complicated by confounding influences such as the presence of additional growth factors, hypoxia, the presence of endotoxins in recombinant proteins, and potential off-target effects of antibodies or soluble receptors, especially when used at high doses.

Taken together, the studies examining rHuEpo induced signaling and related cellular responses in tumors are contradictory and in cases where cellular responses are observed, the responses were modest (2- to 3-fold). The interpretation of these data are further confounded by methodological issues such as use of supraphармacological doses of rHuEpo, the lack of specific inhibitors, and the use of serum free conditions often without appropriate controls.

6.3 Tumor Cell Lines Show No to Minimal Proliferation in Response to rHuEpo in vitro

Studies that assess the biologic response of tumor cell lines to rHuEpo may be more informative than those that measure only intracellular or cell-surface expression of EpoR or EpoR signaling pathways. Investigators have assessed the proliferation of tumor cell lines in response to rHuEpo in vitro in 12 independent studies (Table 16). Eight of the 12 studies reported no increase in proliferation when tumor cell lines were exposed to concentrations of rHuEpo ranging from 0.5 to 5000 U/mL (representing a range of ~1- to 500-fold the typical clinical C_{max} following administration of rHuEpo).

For example, concentrations of rHuEpo up to 100-fold greater (in vitro treatment with rHuEpo up to 1000 U/mL) than those used clinically, did not lead to proliferation of 6 transformed cell lines including those derived from breast, pancreatic, prostate, renal, and myelogenous tumors even though EpoR mRNA and protein was reportedly detected in each cell line using the C-20 polyclonal antibodies (Westphal et al, 2002). Likewise, other investigators (Selzer et al, 2000; Berdel et al, 1991; Berdel et al, 1992; Liu et al, 2004; Mundt et al, 1992; Rosti et al, 1993; Rossler et al, 2004; Dunlop et al, 2006; Gewirtz et al, 2006) did not see a biologic response to rHuEpo as measured by proliferation or clonogenic growth in > 50 independent tumor cell lines exposed to rHuEpo. Although several of these studies did not demonstrate whether

tumor cell lines expressed surface EpoR, one study specifically reported cell lines expressing surface EpoR (assessed by flow cytometry using biotinylated-Epo) that could not be induced to proliferate when exposed to rHuEpo (Liu et al, 2004).

In 4 publications, proliferation of tumor cell lines in response rHuEpo has been reported (Acs et al, 2001; Takeshita et al, 2000; Westenfelder and Barabowski 2000; Feldman et al, 2006). However, the biological significance of these data is uncertain for a number of reasons. First, the increase in proliferation ranging from 1.01- to 4-fold above control levels can be characterized as minimal responses; any response of this magnitude could be within the range of the background noise observed in typical cell proliferation assays. Second, no dose-response relationship was established, as either single concentrations of rHuEpo were used (Takeshita et al, 2000; Acs et al, 2001) or no convincing dose-response was observed over a range of clinically relevant rHuEpo concentrations (Feldman et al, 2006). These data are in contrast to the effect of rHuEpo on an erythroid cell line, in which pharmacologically relevant doses between 0.01 and 0.4 U/mL rHuEpo stimulated a 650% increase in proliferation (Hammerling et al, 1996). Furthermore, the proliferative response observed in the breast tumor MCF-7 line (Acs et al, 2001) was not observed in 5 other studies using the same line (Berdel et al, 1992; Berdel et al, 1991; Mundt et al, 1992; Rosti et al, 1993; Gewirtz et al, 2006).

In summary, the current literature does not support the hypothesis that treatment of tumor cell lines in vitro with rHuEpo leads to an increase in cellular proliferation. In cases where proliferation was reported, methodological limitations or conflicting results from different investigators call into question the validity of the data.

6.4 Tumor Cell Line Survival in Response to rHuEpo Alone or in Combination With Radiation or Chemotherapeutic Agents

Experiments have been conducted to test the hypothesis that ESAs modulate apoptotic responses and/or tumor cell survival in combination with radiation or chemotherapeutic agents in vitro (Table 17). The results from these studies do not support a compelling argument for a biological role of ESAs in modulating tumor cell line survival and/or apoptotic responses in vitro.

Nine independent studies have been reported and 7 showed decreased apoptosis or increased cell survival in response to rHuEpo combined with either radiation or chemotherapeutic agents. However, in all cases the responses were minimal to modest (≤ 50% response relative to controls) and most of the investigators used

suprapharmacological doses of rHuEpo (2.5- to 20-fold over the clinically achievable C_{max}) to induce a biological response (Acs et al, 2003; Acs et al, 2004b; Belenkov et al, 2004; Kumar et al, 2006; McBroom et al, 2005; Pregi et al, 2006). In the studies where clinically relevant concentrations (≤ 10 U/mL) were used, minimal (≤ 20% response relative to controls) or no biological response was observed (Gewirtz et al, 2006; Kumar et al, 2005; Kumar et al, 2006; Liu et al, 2004; McBroom et al, 2005).

For example, rHuEpo (25 to 200 U/mL) in combination with cisplatin was reported to improve HeLa cell survival by approximately 5% to 60% compared with cisplatin alone (Acs et al, 2003). These results are of uncertain significance given that chemotherapy alone typically causes cell death in vitro on a logarithmic scale. As such, a difference of 5% to 60% is difficult to assess. In similar studies, the addition of suprapharmacological concentrations of rHuEpo (30 U/mL) to U87 glioblastoma and HT100 cervical carcinoma tumor cell lines was reported to make the cells more resistant to ionizing radiation and cisplatin (Belenkov et al, 2004). In contrast, other investigators have observed that the magnitude of cell killing by cisplatin in a variety of cell lines pretreated with rHuEpo (10 U/mL) was not reduced when compared with treatment with cisplatin alone (Liu et al, 2004). A similar conclusion was reached by Gewirtz et al (2006) using breast cancer and leukemia cell lines. Although rHuEpo could activate some signaling pathways it did not stimulate proliferation of MCF-7, MDA-MB231, or F-MEL cells and also failed to interfere with antiproliferative, cytotoxic and/or apoptotic effects of several different chemotherapeutic drugs (Gewitrz et al, 2006). Carvalho et al (2005) reported that pharmacologically relevant doses of rHuEpo (4 or 8 U/mL) in combination with daunorubicin or vinblastine increased the apoptotic sensitivity of a renal carcinoma cell line (RCC) and a leukemia cell line (U937).

In conclusion, the hypothesis that rHuEpo interferes with the antiproliferative, cytotoxic and/or apoptotic effects of ionizing radiation or chemotherapeutic drugs is not supported by the currently available in vitro data.

6.5 ESAs in Animal Tumor Models

Limitations exist when using rodent syngenic or xenograft tumor models to assess the effects of various therapies, including ESAs, on tumor growth. Such models do not accurately replicate human tumor growth, because they are initiated from millions of cells (rather than a single cell), they grow to large sizes in a matter of weeks, they depend on an ectopic blood supply, and they must often be grown in

immunocompromised rodents. While each of the studies discussed below has potential aspects that could be criticized, in general they were well designed and executed. Many of them were performed by blinded investigators, had objective endpoints, achieved statistically significant and biologically meaningful results with appropriate numbers of animals, and examined clinically relevant doses of ESAs (ie, increased hematocrit observed).

With the above caveats in mind, none of the rodent tumor studies reported that ESAs alone (23 of 23 studies; Table 18), or in combination with other therapeutic agents, enhanced tumor growth or decreased animal survival. In fact, it is of considerable interest that ESAs restored the effectiveness of radiation therapy, photodynamic therapy, and chemotherapy in anemic animals in numerous tumor xenograft models (Table 18).

For example, SCID mice bearing small, subcutaneous ovarian tumors showed a significant decrease in tumor progression after treatment with rHuEpo plus cisplatin relative to control animals (Silver and Piver, 1999). In animals treated with rHuEpo alone, no increase in tumor growth was observed. In another setting, rHuEpo was shown to induce tumor regression in a murine model of myeloma by provoking an anti-tumor immune response (Mittelman et al, 2001). Daily treatment of tumor-bearing mice with rHuEpo resulted in complete tumor regression in 30% to 60% of the mice. The effect of rHuEpo administration on the course of tumor progression was further investigated using 5T2 MM and 5T33 MM multiple myeloma cells (Mittelman et al, 2003). Treatment was reported to interfere with the proliferation of multiple myeloma cells and significantly prolonged animal survival. These investigators recently reported prolonged survival in 6 patients with advanced multiple myeloma when treated with rHuEpo with or without chemotherapy (Mittelman et al, 2004).

In a nonanemic rat R3230 mammary adenocarcinoma model, administration of 2000 U/kg rHuEpo 3x/week, equivalent to a 60,000 to 100,000 U human dose that is not used clinically, did not lead to enhanced tumor growth (Blackwell et al, 2003). When administered before fractionated radiation, darbepoetin alfa delayed tumor growth in mice 2.7 days as compared with mice treated with radiation alone and 7.3 to 10.6 days as compared with mice receiving darbepoetin alfa alone (Ning et al, 2005). Sigounas et al (2004) treated mice with Lewis lung carcinomas with rHuEpo in combination with cisplatin, mitomycin C, or cyclophosphamide and concluded that rHuEpo treatment synergized with chemotherapeutic agents to further suppress growth of tumors. Others

have explored rHuEpo in combination with fractionated radiation in a murine cancer cachexia model; rHuEpo had a beneficial effect by contributing to radiosensitization and/or reducing weight loss (Pinel et al, 2004; van Halteren et al, 2004). Most studies showed no tumor promoting effect of ESAs including recent ones (Kirkpatrick et al, 2006; Ning et al, 2005; Shannon et al, 2005; LaMontagne et al, 2006, Hardee et al, 2006; Hardee et al, 2005). One group has reported differing results (Kjellen et al, 2006). In this very unusual model, epoetin β alone had no effect on xenograft tumors, but there was increased tumor growth of the head and neck squamous cell carcinoma cell line HNxSCX-7 implanted into mice treated with epoetin β and concomitant mock surgical trauma (a hypodermic needle was drawn through the tumors in situ). It is unclear how much weight to place on this study given the unusual and non-standard methodologies employed, and the larger, contrary body of data.

In summary, in vivo xenograft studies have clearly demonstrated that treatment with ESAs can reduce tumor growth through chemo- or radiosensitization, or enhanced tumor immunity. Most importantly, no evidence exists that treatment with ESAs alone enhances tumor progression or decreases survival in these models.

6.6 Over Production of Epo in Mice and Humans

Biological disorders that alter the levels of Epo in humans and animals provide additional insights into the potential role of ESAs in tumor induction or progression. Primary congenital disorders associated with increased Epo production, or mutations in EpoR leading to hypersensitivity to Epo, are both associated with erythrocytosis in humans (Arcasoy et al, 2002; Gordeuk et al, 2004). However, a greater cancer incidence has not been observed in patients with familial and congenital polycythemia. Transgenic mice have been developed that express high levels of Epo (Madan et al, 2003; Vogel et al, 2003; Wagner et al, 2001). These mice express 10 to 12 times the normal levels of erythropoietin (Ruschitzka et al, 2000). Despite markedly increased endogenous Epo levels, no apparent increase in tumorigenesis was seen in these animals after 2 years of observation. The available information on mice and humans with disorders resulting in chronically increased Epo levels or EpoR sensitivity does not support a tumorigenic effect of ESAs.

7.0 ESAs and Neovascularization

A number of investigators have hypothesized that ESAs might promote tumor angiogenesis through direct endothelial cell stimulation or promote tumor growth by increasing tumor blood vessels through endothelial progenitor cell-mediated vasculogenesis; these hypotheses are addressed below.

7.1 Putative EpoR Expression on Endothelial Cells

EpoR expression has been reported in cultured endothelial cells, as well as in blood vessels from in vivo angiogenesis models and human tissues. This is not unreasonable given the evidence that endothelial cells and erythroid cells arise from a common precursor cell, the hemangioblast, and given the common regulatory mechanisms that appear to be involved in both lineages. However, there are major problems in understanding the biological impact of Epo on the vascular compartment.

The following studies have assessed putative EpoR expression in blood vessels by RT-PCR and/or IHC, and as such, interpretation of these studies is confounded by the limitations of these detection methods (see Sections 0 and 0, above); the results using EpoR antibodies are therefore of dubious significance as they probably reflect expression of non-EpoR proteins. Putative EpoR expression has been observed in neovessels present in explanted human myocardium (Jaquet et al, 2002), chick chorioallantoic membrane (CAM) (Ribatti et al, 1999), tumor xenograft models (Nakamatsu et al, 2004; Yasuda et al, 2003), human umbilical cord (Anagnostou et al, 1994), human placenta (Anagnostou et al, 1994), and primary human tumors (Amin et al, 2005; Arcasoy et al, 2005; Batra et al, 2003; Ribatti et al, 2003; Yasuda et al, 2002).

A number of studies have attempted to correlate Epo or EpoR expression in tumors with tumor microvessel density and other endpoints. A correlation was observed between endogenous Epo content (by radioimmunoassay of tumor lysates) and vascular density in chemically-induced murine hepatic tumors, although no correlation was observed between Epo content and tumor size or between vascular density and tumor size (Nakamatsu et al, 2004). High Epo expression in tumor sections (measured by IHC) was reportedly associated with high microvessel density and better recurrence-free/overall survival in human NSCLC (Amin et al, 2005). However in other studies no correlation was observed between Epo or EpoR expression in tumors, as measured by IHC, and microvessel density in primary human breast cancer samples (Arcasoy et al, 2005). In gastric cancers, increased histologic grade was accompanied by increased

microvessel density, EpoR-positive endothelial cells, and EpoR-positive tumor cells (as measured by IHC using the non-specific Santa Cruz C20 antibodies). The investigators concluded that this was consistent with the hypothesis that Epo has a trophic effect on gastric tumor vasculature (Ribatti et al, 2003). Correlations between Epo or EpoR expression and microvessel density, however, do not prove causation, and microvessel density is not a reliable marker of angiogenesis.

Cultured HUVECs (human umbilical vein endothelial cells), bovine capillary endothelial cells, and rat brain capillary endothelial cells have been shown in radiolabelled rHuEPO binding studies to express between 10,000 and 27,000 low-affinity (1 to 4 nM KD) receptors per cell (Yamaji et al, 1996; Anagnostou et al, 1990), in contrast to erythroid progenitor cells that have lower numbers of EpoRs (135 to 1050 per cell) of high-affinity (100 to 570 pM KD) (Broudy et al, 1991; Sawada et al, 1990; Sawyer et al, 1990). Cross-linking studies further revealed that the receptor that bound to radiolabelled rHuEpo on HUVECs migrated at 45 kDa (Anagnostou et al, 1990), in contrast to the actual size of EpoR (59 kDa) in hematopoietic cells (Laugsch, 2006; Elliott et al, 2006a; Elliott et al, 2006b). It is unclear why these cells would express increased EpoR numbers of much lower affinity. Indeed, the size of the receptor detected calls into question whether EpoR is the receptor being characterized in these studies. Anagnostou et al (1990) speculate that the receptors they detected on endothelial cells are distinct from those on erythroid cells and might play a mitogenic role, rather than a differentiating role, as they do on erythroid cells. A mitogenic effect of rHuEpoR on endothelial cells was observed, but it was modest (see Section 0, below).

In summary, several investigators have reported putative expression of EpoR on endothelial cells and blood vessels. This body of work suffers from the same issues as identified with assessing EpoR on tumor cells and tissues. Although EpoR mRNA can be sensitively and specifically measured by RT-PCR, it is well documented that expression of EpoR mRNA does not necessarily correlate with EpoR protein synthesis and trafficking to the cell surface of a functional EpoR. It has also been shown that there are no reliable methods to detect EpoR protein by IHC due to the non-specific anti-EpoR polyclonal antibodies currently available. Even when more reliable methodologies (^{125}I-binding) to assess EpoR expression on endothelial cells were used, unusually high numbers of low affinity receptors were detected and the identity of these receptors could not be confirmed with cross-linking experiments. Thus, it is still unclear if functional EpoR is expressed on endothelial cells or vasculature.

7.2 Effects of ESAs on Cultured Endothelial Cells

A number of investigators have evaluated the effects of rHuEpo on cultured endothelial cells, using assay endpoints purported to reflect functions required for angiogenesis (ie, migration, differentiation, proliferation, survival, and sprouting). For example, rHuEpo was shown to stimulate cell proliferation in a variety of cultured endothelial cells including immortalized HUVEC-lung cancer chimeric cells, HUVECs, bovine capillary endothelial cells (BACECs), RBECs, and primary rat mesenteric microvascular endothelial cells (Ribatti et al, 1999; Ashley et al, 2002; Haller et al, 1996; Anagnostou et al, 1990; Anagnostou et al, 1990; Yamaji et al, 1996). Suprapharmacological concentrations of rHuEpo have been used on cultured endothelial cells to support a role of the Epo:EpoR axis in various aspects of angiogenesis. Within the range of rHuEpo evaluated in each study, maximal effects were achieved at the concentrations shown in parentheses. Suprapharmacological concentrations of rHuEpo (30 U/mL) caused VEGF release in some cultured tumor cell lines, and the resulting conditioned media induced migration of brain microvascular endothelial cells (Batra et al, 2003). Arroyo et al (1998) observed that rHuEpo (20 U/mL) and VEGF conferred co-mitogenic effects on bovine aortic endothelial cells, although rHuEPO had no effect on its own. rHuEpo enhanced the migration (20 U/mL) of HUVECs and BACECs (Anagnostou et al, 1990). Recombinant HuEpo (100 U/mL) also was shown capable of preventing lipopolysaccharide-induced apoptosis in bovine pulmonary artery endothelial cells (Carlini et al, 1999). These reported concentrations, though, are not attainable in patients. rHuEpo stimulated endothelial cord formation in primary rat mesenteric microvascular endothelial cells (50 U/mL) (Ashley et al, 2002) and Ea.Hy926 cells (2 U/mL) (Ribatti et al, 1999) seeded on Matrigel. Lastly, rHuEpo enhanced capillary sprouting from explanted human myocardium (2.5 U/mL) (Jaquet et al, 2002) and explanted aortic rings (50 U/mL) (Carlini et al, 1995).

Results of all of the above studies should be interpreted with caution, as the fold induction observed is generally minimal (< 2-fold) and the biological relevance of assays performed in isolated endothelial cells is unclear. Moreover, the concentrations of rHuEpo used in most of the studies were very high, 2- to 10-fold higher than concentrations that can maximally be achieved clinically (see Section 6.1), and therefore the results are of dubious significance.

7.3 Effects of ESAs and Epo Inhibitors on Angiogenesis in vivo

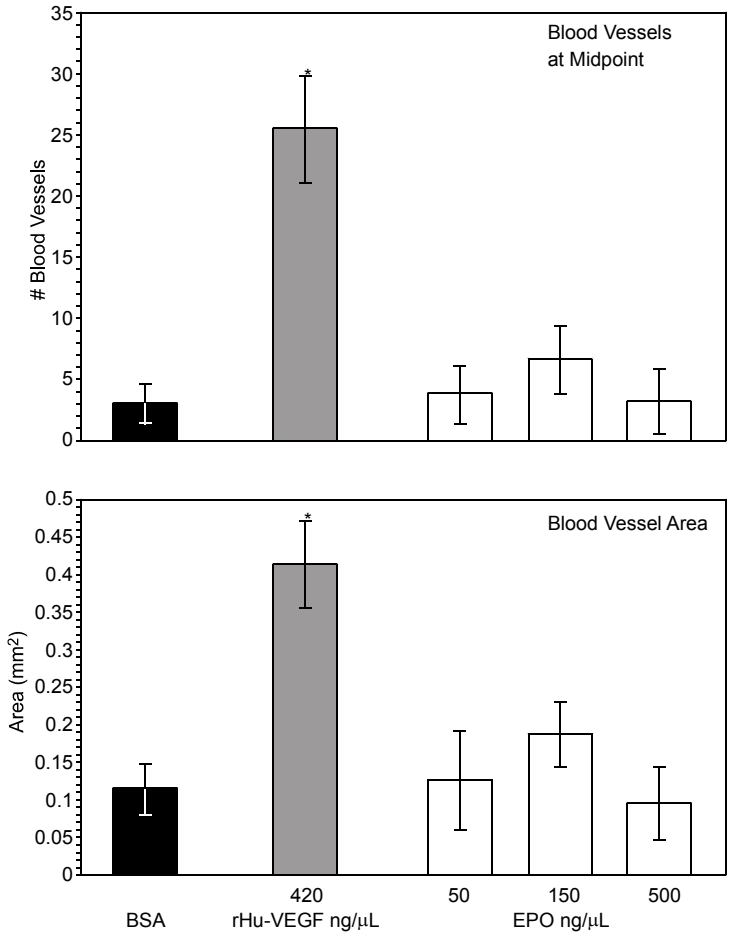
Various angiogenesis models, both within and outside the context of tumor growth, have been used to examine whether rHuEpo or Epo mimetics stimulate neovessel formation in vivo. Direct local injection of rHuEpo into the mouse uterine cavity at very high levels (approximately 2500 U) promoted vessel formation in the endometrium (Yasuda et al, 1998). Recombinant HuEpo was shown to induce chick embryo chorioallantoic membrane angiogenesis (10 U/sponge locally) (Crivellato et al, 2004; Ribatti et al, 1999). Suprapharmacologic levels of locally administered recombinant murine Epo (100 U/mL) or rHuEpo (500 U/mL) enhanced granulation tissue formation and increased microvessel density in a fibrin-induced wound-healing model (Haroon et al, 2003). The very high locally administered doses of rHuEpo call into question the biological relevance of these studies. Systemically-administered rHuEpo was reported to increase microvessel density in preclinical models of myocardial infarction (Hirata et al, 2006; van der Meer et al, 2005). An Epo mimetic peptide reportedly increased microvessel density in tumor xenografts (Yasuda et al, 2003), but no change in hematocrit was observed, suggesting that the effect was not EpoR-mediated.

Four groups of investigators have evaluated the effects of systemically administered rHuEpo on rodent tumor vessels (Ceelen et al, 2007; Hardee et al, 2005; Tovari et al, 2005; Pinel et al, 2004). Recombinant HuEpo did not alter vascular length density in a rat orthotopic mammary carcinoma model (Hardee et al, 2005) or microvessel density in 2 human glioma xenografts (Pinel et al, 2004) or a syngeneic rat colon carcinoma (Ceelen et al, 2007), but it induced microvessel dilatation (Tovari et al, 2005; Ceelen et al, 2007), increased endothelial cell proliferation, and possibly enhanced tumor perfusion in a human epidermoid carcinoma model (Tovari et al, 2005) and increased tumor microvessel permeability in a rat colon carcinoma model (Ceelen et al, 2007). In these latter 2 studies, rHuEpo was also shown to decrease the tumor expression of the pro-angiogenic factor VEGF, perhaps as a result of enhanced tumor oxygenation (Tovari et al, 2005; Ceelen et al, 2007). Despite the apparent vascular alterations described in Tovari et al (2005), rHuEpo had no single-agent effect on tumor growth in either of the 2 models described in this paper or in 7 additional cancer models studied in the other 3 reports. Indeed, when combined with chemotherapy (Tovari et al, 2005) or radiotherapy (Pinel et al, 2004), rHuEpo was shown to confer an enhanced anti-tumor effect. Clearly, studying in isolation the effects

of rHuEpo on tumor vasculature in vivo can be misleading. These studies assessing the impact of rHuEpo on tumor growth in conjunction with vascular changes have demonstrated that rHuEpo had no effect on tumor growth or vascular density even when increased endothelial cell proliferation was observed.

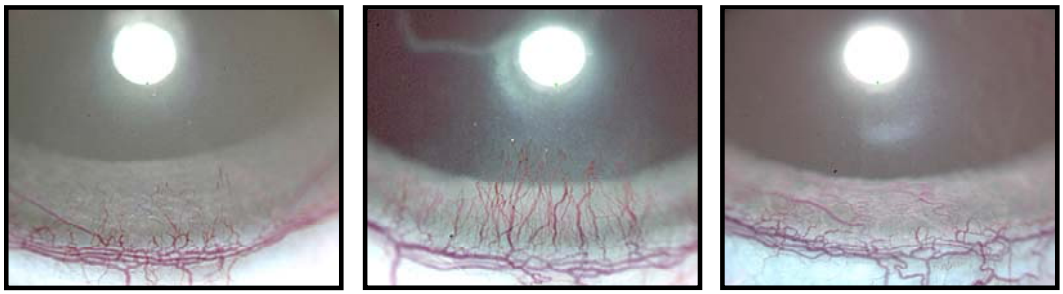
Amgen has not observed any pro-angiogenic effects attributable to murine Epo (mEpo) (Figure 39). Amgen has addressed this question using a rat corneal model of angiogenesis, in which a rHuVEGF or mEpo soaked nylon disk is placed in the avascular cornea at a fixed distance from the surrounding limbal vessels. Neovessels sprouted from the limbal vessels toward the developing VEGF diffusion gradient. In contrast, mEpo, at suprapharmacological concentrations as high as 500 ng/uL (approximately 100,000 U/mL), had no effect on corneal angiogenesis.

Figure 39. Murine Epo does Not Induce Corneal Angiogenesis in Rats



Ang01-10

* p=0.0002 ANOVA with Fisher's



BSA (1000 ng/μl) rHu-VEGF (420 ng/μl) rMu-EPO (500 ng/μl)

Investigation of the potential for murine (m) Epo to induce corneal angiogenesis in a rat model identified there was no effect on increasing blood vessel number or area even at suprapharmacological doses (500 ng/μL) whereas control VEGF (420 ng/μL) had an effect on both parameters. Filter paper disks were soaked in vehicle control (BSA), VEGF or mEpo then placed into a pocket cut into in the cornea. The development of vessels were analyzed after 7 days. Eight rats were used in each study, however 2 rats in the 500 ng/μL mEpo group developed cornel infections and were excluded from the analysis. Representative images of rat corneas from this experiment are shown in the 3-panel image at the bottom of the figure.

Epo and its receptor have been inactivated genetically in a developmental setting (Kertesz et al, 2004). Epo- and EpoR-null mice exhibit angiogenic defects starting at embryonic Day 10.5. These embryos have vascular networks of abnormally low complexity, characterized by diminished capillary sprouting, reduced branching, and decreased vessel diameter. The authors also generated Epo over-expressing transgenic mice, which have abundant tortuous vessels (instead of straight vessels). Crossing the Epo transgenic with the Epo knockout mice partially rescued the Epo-null phenotype. While genetic alterations in the Epo:EpoR pathway have clear vascular phenotypes, the authors could not rule out reduced shear stress (due to decreased hematocrit) as the cause of the angiogenic defects observed in the Epo- and EpoR-deficient mice. Even if the knockout phenotypes reflect a direct effect of Epo on endothelial cells, rather than on hematopoietic cells, it is not clear that the developmental dependency on EpoR signaling for angiogenesis is recapitulated in adult animals or in the setting of malignancy.

7.4 Effect of ESAs on Circulating Endothelial Progenitor Cells (EPC) and Tumor Vasculogenesis

Although angiogenesis is considered the primary process that drives the formation and expansion of the tumor vasculature, more recent evidence suggests that circulating EPCs may contribute to the tumor vasculature. Animal studies have demonstrated that bone-marrow-derived EPCs can differentiate into fully mature endothelial cells and are incorporated at low levels into the tumor vasculature of angiogenesis-competent animals (Natori et al, 2002; Asahara et al, 1999; Ribatti, 2004). The potential for a significant functional contribution of circulating EPCs to tumor vasculature formation and tumor growth was demonstrated most clearly in mutant mice that were angiogenesis-incompetent (Lyden et al, 2001). Angiogenesis-incompetent mice fail to support tumor angiogenesis after tumor challenge. The modest tumor growth observed in the angiogenesis-incompetent mice was accelerated after these same mice were irradiated and received wild type Lac-Z tagged bone marrow. Up to 90% of the tumor vessels found in the angiogenesis-incompetent mice were derived from the LacZ-tagged normal bone marrow. These results indicate that, in the angiogenesis-incompetent mouse, bone-marrow-derived EPCs could provide for the development of a significant tumor vasculature that promoted tumor growth (Lyden et al, 2001).

The functional contribution of EPCs to the tumor vasculature and tumor growth in angiogenesis-competent animals is, however, much less clear. Limited focal

incorporation of EPCs into tumor blood vessels (Asahara et al, 1999; Natori et al, 2002; Machein et al, 2003) has been observed. Both VEGF and PlGF increased bone-marrow derived EPC incorporation 4-fold into murine tumor vasculature over the short term (5 days post treatment) but not the long term (25 days post treatment) (Li et al, 2006). However, others have shown that engineered bone marrow engrafted into angiogenesis-competent irradiated mice did not contribute detectable numbers of EPC to syngeneic tumor endothelium (Gothert et al, 2004). Whether human EPCs significantly contribute to the tumor vasculature of primary or metastatic lesions found in cancer patients is unknown, but there is little convincing evidence that the progenitor cells play a meaningful role in tumor vasculature.

Several investigators have measured circulating EPCs in a variety of cardiovascular disease models and in anemic patients in response to administration of rHuEpo. The 2- to 4-fold increase in EPCs observed in preclinical models were modest, and required suprapharmacological concentrations of ESAs to evoke the response. For example, a 2-fold increase in circulating EPCs (CD34+, VEGFR2+) was reported in normal mice after 3 days of dosing rHuEpo at 1000ug/kg per day and a 3-fold EPC increase in mice with hind limb ischemia after administration of multiple doses over 3 weeks of 1000 U/kg rHuEpo (resulted in serum levels of 40.6 U/mL, 4-fold greater than maximally attainable clinical concentrations) (Heeschen et al, 2003). The authors noted the hind limb ischemia alone increased baseline levels of EPCs which were then further augmented by rHuEpo administration. In rat and dog myocardial infarction models ESAs modestly increased low numbers of circulating EPC levels (Hirata et al, 2006; Prunier et al, 2007). In a hypoxia-induced mouse pulmonary hypertension model, in wild-type mice where endogenous Epo/EpoR interaction remained intact, there appeared to be a modest increase in mobilization and recruitment of EPCs (FLK1+/CD133+) to hypoxic pulmonary tissue (1% increased to 4%) that was not observed in EpoR -/- rescued mice (Satoh et al, 2006). Additionally, rHuEpo therapy increased circulating EPC (CD34+,CD45+) levels in patients with renal-failure-induced anemia by approximately 3-fold as early as 2 weeks after 5000 U/kg/week dosing to the EPC levels observed in healthy nonanemic volunteers (Bahlmann, 2004). In this study the investigators failed to carefully compare the number of EPCs identified by flow cytometry to the total number of cells analyzed per sample or per high powered field making it difficult to interpret these results. In a small set of breast cancer patients, taxane-based neoadjuvant therapy increased circulating EPC levels (CD34+/VEGFR2+) almost 2-fold with a concomitant

nearly 2-fold increase in serum Epo, along with smaller increases in VEGF and Ang2 levels, causing the authors to speculate on an association between increased Epo and increased EPC levels (Fürstenberger et al, 2006).

Marginal effects of ESAs on EPC levels similar to those reported above are likely to have negligible clinical relevance. The most studied and best characterized mobilizer of EPCs in vivo, VEGF, has been reported to increase circulating EPC numbers at least 10-fold (Hattori et al, 2001). This is more consistent with changes of clinical relevance. Circulating EPCs are a subset of CD34-positive blood progenitor cells which have been the focus of clinical investigation for many years. The inter-individual variation in levels of these progenitor cells is over 100-fold for untreated individuals (Begley et al, 1997; Roberts et al, 1995), and the increase seen with other growth factors is of the order of 100-fold (Begley et al, 1997). Thus, changes of 2 to 4-fold likely reflect normal inter-individual variation.

When viewed in aggregate relative to the suprapharmacological concentrations used and the minimal biological effects observed, these studies do not demonstrate any meaningful effect of ESAs on mobilization of EPCs, particularly in relationship to tumor progression. It is also unclear from the currently available literature how significant a role EPCs play in tumor vasculogenesis and if there is any effect of ESAs on tumor angiogenesis.

8.0 ESAs and Tumor Hypoxia

Tumor hypoxia is well known to be an important adverse factor for clinical outcomes as is the beneficial effects of oxygen (Connell et al, 2001). The oxygen enhancement ratio (OER) has been established to be 2.5 to 3.5 (differs for different cell types) which means that 3 times as much radiation is required under hypoxic conditions as under oxic conditions for tumor ablation. More recently however, there has been an understanding of the molecular basis of the adverse effects associated with tumor hypoxia. The cellular response to hypoxia includes significant changes in gene expression to contribute to cellular resistance to radiation therapy and chemotherapy. There have been numerous attempts to take advantage of this knowledge by attempting to mimic the beneficial effects of oxygen using small molecules (“hypoxic cell sensitizers” - the subject of over 240 publications since 1975 source PubMed) and on focusing on the molecules that are triggered by tumor hypoxia as legitimate anti-cancer targets in their own right. This includes targeting the transcriptional regulators of hypoxic genes (eg, HIF-1α) and the

regulation of downstream effecter proteins such as VEGF in angiogenesis (reviewed by Powis & Kirkpatrick, 2004; Milkiewicz et al., 2006). While hypoxia may contribute to tumor progression it does not appear to be relevant in tumor initiation, a time at which cells are not hypoxic; oxygen diffusion probably only becomes limiting with a cell mass of > 1 cm.

Several recent studies in patients with head-and-neck or cervical cancer have confirmed that tumor hypoxia is a powerful prognostic factor associated with malignant progression, metastasis, decreased local tumor control, lower rates of disease-free survival, overall survival, and poor outcome (Harrison et al, 2002; Vaupel et al, 2001; Brizel et al, 1997; Hockel et al, 1996; Vaupel, 2004). Tumor hypoxia, which is potentiated by concomitant anemia, has long been known to render tumors more resistant to radiation and some forms of chemotherapy (Leyland-Jones et al, 2003; Harrison et al, 2002; Vaupel et al, 2001; Tatum et al, 2006). Hypoxic regions in tumors have been described in a myriad of tumor types. Whether treated by surgery or radiotherapy, patients with tumors with lower pO_2 were found to have significantly worse disease-free and overall survival, largely because of locoregional failures (Hockel et al, 1996).

Investigators have assessed the effects on hypoxia on tumor cell lines in vitro (Acs et al, 2004; Kumar et al, 2005; Kumar et al, 2006; Table 17). For example, Acs et al 2004 observed that rHuEpo (200 U/mL) increased survival of MCF-7 breast cancer cells by 30% when exposed to severe hypoxia (< 0.005% pO_2), while similar responses were not seen with moderate hypoxia (1% pO_2). Similar experiments using WM35 melanoma cells under hypoxic conditions after treatment with 10 U/mL rHuEpo showed minimal effects (10%) on survival and no effect under normoxic condition (Kumar et al, 2006). It is unclear how to translate these in vitro findings to in situ tumor hypoxia, where different parts of a tumor may have differing hypoxic states. Other investigators have assessed the impact of ESAs on hypoxia-induced VEGF expression. In addition to regulating VEGF expression, hypoxia also regulates cellular VEGFR2 expression. Initiation of tumor angiogenesis occurs when cells within the tumor microenvironment sense hypoxia and begin to produce VEGF (Semenza, 2001). When grown under aerobic conditions, D12-melanoma cells express low levels of VEGF. Placement of D12-cells under low-oxygen conditions up-regulates their VEGF production, increases tumor angiogenesis, and increases metastatic growth in a mouse model (Leyland-Jones et al, 2003). Human serum VEGF levels have been observed to

decrease in concert with increasing hemoglobin concentrations after rHuEpo therapy (Dunst et al, 2002). As oxygen tension is increased in the tumor microenvironment, not only expression of VEGF, but also other hypoxia-induced genes, including Epo and IGF-2 are reduced (Leyland-Jones et al, 2003; Harris, 2002). Culture of SKOV-3, a human ovarian tumor cell line, with rHuEpo (range 10 to 500 U/mL), decreased both hypoxia induced HIF-1α and VEGF protein expression without altering cellular growth rate (Hale et al, 2006). Thus, erythropoietin-mediated reduction in tumor hypoxia is predicted to significantly decrease tumor angiogenesis, tumor growth, and tumor metastasis.

More importantly, rHuEPO improves tumor oxygenation in xenograft models (Kelleher et al, 1996; Pinel et al, 2004) and may even improve tumor oxygenation independent of its effect of raising hemoglobin levels (Blackwell et al, 2003). Darbepoetin alfa corrected anemia in tumor-bearing mice and sensitized tumor cells to radiation therapy. It also sensitized tumors to radiation before an effect on hemoglobin levels was observed (Ning et al, 2005). The tumor sensitization effect was not observed in non-anemic rat tumor models (Kirkpatrick et al, 2006). Thus, ESAs enhanced the efficacy of radiation therapy in anemic rodent models and may also exert effects through additional undefined mechanisms that are independent of the correction of anemia and/or tumor hypoxia.

In summary, correction of tumor hypoxia by administration of rHuEpo in rodent tumor models has been shown in some cases to increase the sensitivity of tumors to chemo- or radiation therapies and had no impact on tumor growth when administered alone. In addition, correction of hypoxia after ESA treatment decreases VEGF expression which in turn could potentially decrease tumor angiogenesis and growth.

9.0 Conclusions

Numerous investigations have reported putative EpoR expression in tumors and on tumor vasculature and have assumed a negative impact on tumor progression and survival in cancer patients. However, many of the findings and conclusions of these studies are questionable due to confounding factors with methods used to detect EpoR protein, lack of appropriate controls, and lack of detection of physiologically relevant surface EpoR on tumor or endothelial cells. Functional studies that report ESAs confer a proliferative or survival advantage for tumor cells are also conflicting and lack compelling data. For tumor or endothelial cell lines that responded to rHuEpo in vitro, the response was marginal and/or suprapharmacological levels of rHuEpo were required to evoke a

biologic response. In contrast, several studies have demonstrated that many tumor cell lines do not respond to rHuEpo even when EpoR is expressed. These findings may reflect a possible absence of intracellular signaling after ligand-receptor interaction, low EpoR affinity, or non-functional EpoR at the cell surface in tumor cell lines. Of most physiological relevance are the in vivo tumor studies, some of which have shown that treatment with ESAs reduced tumor growth through radiosensitization, reduced hypoxia, or enhanced tumor immunity. Most importantly, in vivo tumor studies have shown that treatment with ESAs do not enhance tumor progression directly or through enhanced angiogenesis/vasculogenesis or decrease animal survival.

Table 15. Tumor Tissue Expression of EpoR Detected by Various Methods

Reference	Tumor Type(s)	Method of Detection	Results
Eccles et al, 2003	Papillary thyroid carcinoma	IHC (C-20 Ab)	11/17 samples expressed EpoR
Abdalla et al, 2005	B-CLL, MCL, and MM	RT-PCR, flow cytometry	32/41 B-CLL and 5/7 MCL and 13/24 MM samples expressed EpoR by RT-PCR; 0/9 B-CLL expressed surface EpoR and 8/8 B-CLL and 4/4 MCL expressed cytoplasmic EpoR
Acs et al, 2001	Breast lobular carcinoma, ductal carcinoma, ovarian cancer, lung cancer, medulloblastoma	IHC, Western (C-20 Ab)	All samples expressed EpoR
Acs et al, 2002	Invasive mammary carcinoma, in situ mammary carcinoma	IHC (C-20 Ab and Upstate Ab)	340/342 samples expressed EpoR
Acs et al, 2003	Benign, dyplastic cervical squamous epithelia and ISCC of the cervix	IHC, Western (C-20 Ab)	All samples expressed EpoR by IHC; 7/7 ISCC expressed EpoR by Western
Acs et al, 2004	Endometrial carcinoma	IHC (C-20 Ab)	107/107 expressed EpoR
Arcasoy et al, 2002b	Primary breast tumor biopsies	IHC (GI Ab)	9/10 tumors (21/26 biopsies) expressed EpoR
Arcasoy et al, 2005a	Squamous cell carcinoma of head and neck	IHC (C-20 and GI Abs)	20/20 expressed EpoR
Arcasoy et al, 2005b	Prostate cancer	IHC (C-20 Ab)	18/18 expressed EpoR
Batra et al, 2003	Pediatric solid tumors	RT-PCR and IHC (SC Ab)	23/24 samples expressed EpoR mRNA and 18/18 expressed EpoR protein

ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; IHC = immunohistochemistry; RT-PCR = reverse transcription-polymerase chain reaction; ISCC = invasive squamous cell carcinoma; C-20 Ab = anti-human EpoR rabbit polyclonal antibody clone C20 (Santa Cruz Biotech); GI Ab = mh2er16.5.1 antibody (Genetics Institute); Calbio Ab = sheep polyclonal anti-human EpoR (Calbiochem); ? = antibody used not clearly defined; Upstate Ab = rabbit polyclonal EpoR; SC Ab = anti-human EpoR rabbit polyclonal antibody (specific clone not identified; Santa Cruz Biotech)

Table 15. Tumor Tissue Expression of EpoR Detected by Various Methods

Reference	Tumor Type(s)	Method of Detection	Results
Dagnon et al, 2005	Lung squamous cell carcinomas and adenocarcinomas	RT-PCR and IHC (C-20 Ab)	5/5 samples expressed EpoR mRNA and 23/24 samples expressed EpoR protein
Dillard et al, 2001z	Vestibular Schwannoma	IHC (SC Ab)	9/14 samples expressed EpoR
Gong et al, 2006	Sporadic clear cell renal cell carcinoma	RT-PCR, Western blotting and IHC (?)	10/10 samples expressed EpoR mRNA and protein by Western; 50/54 samples expressed EpoR by IHC
Henke et al, 2006	Advanced carcinoma of head and neck	IHC (C-20 Ab)	104/154 samples expressed EpoR
Hoogsteen et al, 2005	Squamous cell carcinoma of head and neck	IHC (SC Ab)	80/85 samples expressed EpoR
Kase et al, 2006	Merkel cell carcinoma	IHC (SC Ab)	3/3 samples expressed EpoR
Lai et al, 2005	Squamous cell carcinoma of head and neck	RT-PCR	12/12 primary tumors and 12/12 LN metastases expressed EpoR mRNA
Leo et al, 2006	Cervical cancer	IHC (C-20 Ab)	44/48 samples expressed EpoR
McBroom et al, 2005	Ovarian cystadenomas, serous LMP tumors, serous carcinoma	IHC (C-20 Ab)	EpoR expression observed (incidence not reported)
Mohyeldin et al, 2005	Squamous cell carcinoma of head and neck	IHC (C-20 and Upstate Abs)	32/32 samples expressed EpoR
Pollio et al, 2005	Uterine leiomyomas	IHC (C-20 Ab)	11/17 samples expressed EpoR
Ribatti et al, 2003b	Gastric adenocarcinoma	IHC (C-20 Ab)	EpoR expression observed (incidence not reported)

ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; IHC = immunohistochemistry; RT-PCR = reverse transcription-polymerase chain reaction; ISCC = invasive squamous cell carcinoma; C-20 Ab = anti-human EpoR rabbit polyclonal antibody clone C20 (Santa Cruz Biotech); GI Ab = mh2er16.5.1 antibody (Genetics Institute); Calbio Ab = sheep polyclonal anti-human EpoR (Calbiochem); ? = antibody used not clearly defined; Upstate Ab = rabbit polyclonal EpoR; SC Ab = anti-human EpoR rabbit polyclonal antibody (specific clone not identified; Santa Cruz Biotech)

Table 15. Tumor Tissue Expression of EpoR Detected by Various Methods

Reference	Tumor Type(s)	Method of Detection	Results
Takeshita et al, 2000	AML and ALL	Flow Cytometry (biotinylated Epo)	81/136 AML and 4/14 ALL samples expressed EpoR
Vogel et al, 2005b	Endolymphatic sac tumors	RT-PCR, Western blotting and IHC (Calbio Ab)	5/5 samples expressed EpoR mRNA and EpoR protein
Vogel et al, 2005a	Pheochromocytoma	RT-PCR, Western blotting and IHC (Calbio Ab)	10/10 samples expressed EpoR mRNA and EpoR protein
Vortmeyer et al, 2003a	Hemangioblastomas	RT-PCR, Western blotting and IHC (Calbio and SC Abs)	6/6 samples expressed EpoR mRNA and EpoR protein
Westenfelder and Baranowski, 2000	Renal cell carcinoma	RT-PCR and Western blotting (SC Ab)	3/3 samples expressed EpoR mRNA and 1/1 samples expressed EpoR protein
Winter et al, 2005	Squamous cell carcinoma of head and neck	RT-PCR and IHC (C-20 Ab)	146/146 samples expressed EpoR protein mRNA and 12/16 EpoR protein
Yasuda et al, 2002	Ovarian, endometrial and cervical cancers	RT-PCR and IHC (?)	12/16 tumor specimens expressed EPO-R

ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; IHC = immunohistochemistry; RT-PCR = reverse transcription-polymerase chain reaction; ISCC = invasive squamous cell carcinoma; C-20 Ab = anti-human EpoR rabbit polyclonal antibody clone C20 (Santa Cruz Biotech); GI Ab = mh2er16.5.1 antibody (Genetics Institute); Calbio Ab = sheep polyclonal anti-human EpoR (Calbiochem); ? = antibody used not clearly defined; Upstate Ab = rabbit polyclonal EpoR; SC Ab = anti-human EpoR rabbit polyclonal antibody (specific clone not identified; Santa Cruz Biotech)

Table 16. *In vitro* Proliferation Studies Using rHuEPO

Cell lines	Tissue origin	ESP dose	Result	Study
81 AML and 5 ALL patient samples	Blood	1 U/mL	1.15 fold for AMG and 1.01 for ALL increased proliferation (NS)	Takeshita et al, 2000
MCF-7, BT-549	Breast	10 U/mL	1.25 fold increased proliferation	Acs et al, 2001
FO-1, SK-MEL28, PLC, LXF-289, KTCTC-1M, KTCTL-26A, KTCTL-30, MCF-7, MDA-MB-231	Skin, kidney, mammary, liver, colon, lung	1 to 5000 U/mL	No proliferation	Mundt et al, 1992
H69, N417, OCUM-1, GBLHU2, MCF-7, HL-50, KG1, HEL, PLB-985, K562	Lung, stomach, brain, breast, blood	0.5 to 5 U/mL	No colony stimulating effect	Pedrazzoli et al, 1992a
HTB-43, CCL-23, CCL-17, HTB-119, HTB-120, HTB-19, HTB-22, KATO, HTB-38, WiDr, CCL 187, HepG2, MIA PaCa, HTB-77, HTB-36, CRL-1427, U87MG, 87HG31, CCL-127, B, HTB-45, HTB-44	Head/neck, lung, breast, stomach, GI, liver, pancreas, ovary, brain, prostate, kidney	0.01 to 100 U/mL	No colony stimulating effect	Berdel et al, 1991
K562, HEL, HL-60, KG-1, PLB-985, H69, N417, MCF-7, OCUM-1, GBL-HU12	Blood, lung, breast, stomach, brain	0.5 to 10 U/mL	No colony stimulating effect	Rosti et al, 1993
K562, HepG2, KTCTL-30, NMB, S117, RT112	Blood, liver, kidney, neural, thyroid, bladder	10 to 1000 U/mL	No proliferation	Westphal et al, 2002
Caki-2, 786-0, RAG 3	Kidney	0.5 to 100 U/mL	1.25 to 4 fold increased proliferation	Westenfelder and Baranowski, 2000
ACHN, Caki 1, CEM, HCT116, HL60, K567, U266	Renal, blood, GI	5 to 20 U/mL	No proliferation	Liu et al, 2004
H838	Lung	12.5 U/mL	No proliferation	Dunlop et al, 2006
SH-SY5Y, LAN-5, Kelly, SK N LO, KS-N-MC and SH-SHEP	Brain	1.25 mU/mL to 10 U/mL	No proliferation	Rossler et al, 2004
PC3, LNCaP, 267B1, X/267B1, Ki/267B1	Prostate	1 to 100 U/mL	1.4 to 3.5 fold increased proliferation 4/5 lines	Feldman et al, 2006

NS indicates not statistically significant; ALL, acute lymphoblastic leukemia; and AML, acute myeloblastic leukemia.

Table 17. *In vitro* Survival Studies Using rHuEPO in Combination With Hypoxia, Radiation, or Chemotherapy

Cell lines	Tissue origin	ESP dose/other treatments	Result	Study
HeLa	Cervix	25, 50, 100, 200 U/mL +/- cisplatin	Increased survival and reduced apoptosis	Acs et al, 2003
MCF-7	Breast	200 U/mL +/- hypoxia	Under severe hypoxia reduced apoptosis and increased survival	Acs et al, 2004
U87 and HT100	Brain and cervix	30 U/mL +/- cisplatin or radiation	Increased survival in response to cisplatin or radiation	Belenkov et al, 2004
RCC and U937	Kidney and blood	4 or 8 U/mL +/- daunorubicin or vinblastine	Increased apoptotic response to CT drugs	Carvalho et al, 2005
MCF-7, MDA-MB231 and F-MEL	Breast and blood	10 U/mL +/- adriamycin, taxol, tamoxifen, cytarabine or daunorubicin	No proliferation. Did not interfere with antiproliferative, cytotoxic and/or apoptotic effects of CT drugs	Gewirtz et al, 2006
WM35, WM793 and 1205 Lu	Melanoma cells	10 or 100 U/mL +/- hypoxia or DITC or cisplatin	Increased resistance to moderate hypoxia and CT drugs	Kumar et al, 2005
WM35	Melanoma cells	10 U/mL +/- hypoxia	Increased survival under hypoxic conditions but not normoxic conditions	Kumar et al, 2006
ACHN, Caki 1, CEM, HCT116, HL60, K567, U266	Renal, blood, GI	10 U/mL +/- cisplatin	No increase in viability +/- cisplatin	Liu et al, 2004
OVCAR3 and SKOV3	Ovary	1, 10, 100, 200 U/mL +/- cisplatin	Iincreased resistance to CT drugs in 1 of 2 cell lines	McBroom et al, 2005
HeLa (tranfected with inducible EpoR construct)	Cervix	25 U/mL +/- radiation	Increased clonogenicity of transfected cells. No effect on radiation sensitivity	Pajonk et al, 2004
SH-SY5Y	Brain	25 U/mL +/- staurosporine	Increased resistance to staurosporine induced apoptosis	Pregi et al, 2006

NS indicates not statistically significant; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; and CT, chemotherapy

Table 18. Effect of ESAs in Xenograft or Syngeneic Tumor Models

Tumor type and Origin	ESA Dose	Tumor and survival outcomes	Study
Rat DS-Sarcoma	1,000 U/kg Epo TIW	No effect.	Kelleher et al, 1996
Murine MmB16 melanoma	20 U Epo b.i.d	No effect alone. No enhanced IL-12 therapy.	Golab et al, 1998
Rat DS-sarcoma	1,000 U/kg	No effect alone. Improved ablative RT.	Thews et al, 1998
Ovary adenocarcinoma	20 U Epo TIW	No effect alone. Improved CT.	Silver and Piver, 1999
Murine myelomas MOPC-315, 5T33 MM	30 U Epo QD	Tumor regression and prolonged survival.	Mittleman et al, 2001
Glioblastoma HTZ II	1,000 U/kg Epo TIW	No effect alone. Improved RT in anemic mice.	Stuben et al, 2001
Rat DS-sarcoma	1,000 U/kg Epo TIW	No effect alone. Improved CT in anemic rats.	Thews et al, 2001
Colon adenocarcinoma	1,000 U/kg Epo QD	Restored PT in anemic mice.	Golab et al, 2002
Rat R3230 mammary carcinoma	2,000 U/kg Epo TIW	No effect alone.	Blackwell et al, 2003
Rat R3230 mammary carcinoma	3 µg/kg DA TIW	No effect to enhance RT.	Kirkpatrick et al, 2006
Murine BCL-1 leukemia/lymphoma	30 U Epo QD	Tumor regression and prolonged survival.	Mittleman et al, 2003
Neurogenic sarcoma ENE2	750 U/kg Epo TIW	Improved RT therapy in anemic mice	Stuben et al, 2003
Lewis lung carcinoma	60 U/kg Epo (2 doses)	No effect alone. Enhanced CT.	Sigounas et al, 2004
Murine C26-B adenocarcinoma	25 U Epo QD to 25 U TIW per mouse	No effect on tumor. Decreased body weight loss.	Van Halteren et al, 2004
Human glioblastomas GBM, Nan1 and U87	300 U/kg QD	No effect on tumor alone. Enhanced RT.	Pinel et al, 2004

TIW indicates 3 times per week; b.i.d., twice per day; QD, once daily; QW, once per week; Q2W, every 2 weeks; RT, radiotherapy; CT, chemotherapy; PT, photodynamic therapy; and DA, darbepoetin alfa.

Table 18. Effect of ESAs in Xenograft or Syngeneic Tumor Models

Tumor type and Origin	ESA Dose	Tumor and survival outcomes	Study
Murine SCC VII squamous cell carcinoma and RIF-1 fibrosarcoma	30 µg/kg DA QW or Q2W	No effect alone. Improved RT in anemic mice.	Ning et al, 2005
Lewis lung carcinoma	10 µg/kg DA QW	No effect alone. Improved CT.	Shannon et al, 2005
Rat R3230 mammary carcinoma, murine CT26 colon carcinoma, human HCT-116 colon carcinoma, human FaDu head and neck carcinoma	2,000 U/kg Epo TIW	No effect alone.	Hardee et al, 2005
Rat R3230 mammary carcinoma	2000 U/kg Epo TIW	No effect alone or with Taxol	Hardee et al, 2006
Human breast carcinomas MDA-MB-231 and MCF-7	0.0025 mg/kg Epoetin α, 0.0075 mg/kg DA, and 0.0025 mg/kg Epoetin β	No effect alone or with paclitaxel	LaMontagne et al, 2006
Head and neck squamous cell carcinoma LU-HNxSCX-7	400 U/kg Epoetin β Q3D	No effect alone. Increased tumor growth with mock surgical transaction.	Kjellen et al, 2006
Rat 13762 NF mammary adenocarcinoma	50 ug/kg Epoetin α TIC	No effect alone or with cisplatin. Partially prevented cisplatin induced peripheral neurotoxicity.	Bianchi et al, 2007
Human squamous cell A431, colorectal carcinoma HT25	150 U/kg Epo TIW	No effect alone. Enhanced CT.	Tovari et al, 2005

TIW indicates 3 times per week; b.i.d., twice per day; QD, once daily; QW, once per week; Q2W, every 2 weeks; RT, radiotherapy; CT, chemotherapy; PT, photodynamic therapy; and DA, darbepoetin alfa.

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Appendix 3A. Amgen-sponsored Studies Included the Combined Analyses: Chemotherapy-induced Anemia

Study Number (n)[a]	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
Randomized Placebo-controlled, Phase 3				
980297 (n = 314)	A double-blind, placebo-controlled, randomised study of novel erythropoiesis stimulating protein (NESP) for the treatment of anaemia in lung cancer subjects receiving multicycle platinum-containing chemotherapy	2.25 μg/kg QW	Placebo	12 weeks
20000161 (n = 344)	A multicenter, blinded, placebo-controlled, randomized study of novel erythropoiesis stimulating protein (NESP) for the treatment of anemia in subjects with lymphoproliferative malignancies receiving chemotherapy	2.25 μg/kg QW	Placebo	12 weeks
20010145[b] (n = 583)	A randomized, double blind, placebo-controlled study of subjects with previously untreated extensive-stage small-cell lung cancer (SCLC) treated with platinum plus etoposide chemotherapy with or without darbepoetin alfa	300 μg QW followed by 300 μg Q3W	Placebo	16 weeks
20030232 (n = 386)	A randomized, double-blind, placebo-controlled study of darbepoetin alfa for the treatment of anemia in subjects with non-myeloid malignancy receiving multicycle chemotherapy	300 μg Q3W	Placebo	15 weeks
Randomized Placebo-controlled, Phase 2				
980291 Schedule 1 (n = 249)	A randomized, double-blind, placebo-controlled, dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous (SC) injection for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy	4.5, 6.75, 9.0, 12.0, 13.5, 15.0 μg/kg Q3W	Placebo	12 weeks
Schedule 2 (n = 156)		9.0, 12.0, 15.0, 18.0 μg/kg Q4W	Placebo	12 weeks
990114 (n = 66)	A multi-centre, blinded, placebo-controlled, randomized, dose finding study of NESP administered by SC injection for the treatment of anaemia in subjects with lymphoproliferative malignancies receiving chemotherapy	1.0, 2.25, 4.5 μg/kg QW	Placebo	12 weeks

Appendix 3A. Amgen-sponsored Studies Included the Combined Analyses: Chemotherapy-induced Anemia

Study Number (n)[a]	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
Randomized Front Loading DA vs. Active Control, Phase 3				
20020118 (n = 723)	A randomized, double-blind, study of front-loading darbepoetin alfa compared with standard weekly administration for the treatment of anemia in subjects with a non-myeloid malignancy and receiving multicycle chemotherapy	4.5 μg/kg QW followed by 4.5 μg/kg Q3W	DA 2.25 μg/kg QW	16 weeks
20010101 (n = 699)	A randomized, open-label study of darbepoetin alfa (novel erythropoiesis stimulating protein, NESP) and rHuEPO for the treatment of anemia in subjects with non-myeloid malignancies receiving multicycle chemotherapy	4.5 μg/kg QW followed by 4.5 μg/kg Q3W	rHuEPO 150 U/kg TIW	16 weeks
20020139 (n = 703)	A randomized, open-label study to assess time to hemoglobin response of front load dosing regimen for darbepoetin alfa compared to a weekly dose regimen for recombinant human erythropoietin in patients with non-myeloid malignancies receiving chemotherapy	4.5 μg/kg QW followed by 4.5 μg/kg Q3W	rHuEPO 40,000 U QW	12 weeks
Randomized Front Loading DA vs. Active Control, Phase 2				
20000174 (n = 122)	A dose- and schedule-finding study of novel erythopoiesis stimulating protein (NESP) for the treatment of anemia in subjects with solid tumors receiving chemotherapy	4.5 μg/kg QW followed by 1.5 μg/kg QW, 2.25 μg/kg QW, or 3.0 μg/kg Q2W	rHuEPO 40,000 U QW	12 weeks
Randomized Less Frequent DA vs. Active Control, Phase 3				
20030231 (n = 705)	A randomized, double-blind, active-controlled study of darbepoetin alfa for the treatment of anemia in subjects with non-myeloid malignancy receiving multicycle chemotherapy	500 μg Q3W	DA 2.25 μg/kg QW	15 weeks
20030125 (n = 1209)	A randomized, open-label, multicenter study of darbepoetin alfa administered once every 2 weeks (Q2W) compared with Epoetin alfa administered once every week (QW) for the treatment of anemia in subjects with non-myeloid malignancies receiving multicycle chemotherapy	200 μg Q2W	rHuEPO 40,000 U QW	16 weeks

Appendix 3A. Amgen-sponsored Studies Included the Combined Analyses: Chemotherapy-induced Anemia

Study Number (n)[a]	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
Randomized Less Frequent DA vs. Active Control, Phase 2				
20020152 (n = 141)	An open-label, randomized phase 2 study to validate a patient satisfaction questionnaire for anemia treatment in female breast cancer patients treated with darbepoetin alfa or recombinant human erythropoietin for anemia due to chemotherapy	200 μg Q2W	rHuEPO 40,000 U QW	16 weeks
20020165 (n = 102)	An open-label, randomized phase 2 study to validate a patient satisfaction questionnaire for anemia treatment in patient with non-small cell lung cancer treated with darbepoetin alfa or recombinant human erythropoietin for anemia due to chemotherapy	200 μg Q2W	rHuEPO 40,000 U QW	16 weeks
20020166 (n = 69)	An open-label, randomized phase 2 study to validate a patient satisfaction questionnaire for anemia treatment in patients with gynecological malignancies treated with darbepoetin alfa or recombinant human erythropoietin for anemia due to chemotherapy	200 μg Q2W	rHuEPO 40,000 U QW	16 weeks
20040262 (n = 752)	Flexibility: A Study to Assess the Impact of Once per Cycle Correction and Maintenance Dosing of Darbepoetin Alfa in Subjects with Non-myeloid Malignancies with Anemia Due to Chemotherapy	300 or 500 μg Q3W	DA 150 μg QW	24 weeks
980290B (n = 160)	A randomized dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous injection for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy	3.0, 5.0, 7.0, 9.0 μg/kg Q2W	rHuEPO 40,000 U QW	12 weeks

[a] Number of subjects randomized who received at least 1 dose of investigational product

Appendix 3B. Amgen-sponsored Study Included in the Combined Analyses: Active Cancer Not Receiving Chemotherapy or Radiotherapy

Study Number (n)[a]	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
Randomized, Placebo-controlled, Phase 3				
20010103 (n = 989)	A multicenter, randomised, double-blind, placebo-controlled study of darbepoetin alfa for the treatment of anemia of cancer	6.75 µg/kg Q4W	Placebo	16 weeks

[a] Number of subjects randomized who received at least 1 dose of investigational product

[b] Planned enrollment

Appendix 4A. Amgen-sponsored Studies Not Included in a Combined Analysis: Chemotherapy-induced Anemia

Study Number (n)[a]	Phase	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
980290A (n = 269)	1/2	A randomized dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous injection for the treatment of anemia in subjects with solid tumors receiving multicycle chemotherapy	0.5, 1.0, 1.5, 2.25, 4.5, 6.0, 8.0 μg/kg QW	rHuEPO 150 U/kg TIW	12 weeks
20000220 (n = 1558)	2	An open-label, randomized study to develop a screening tool for functional capacity in anemic subjects with nonmyeloid malignancies receiving chemotherapy and darbepoetin alfa	3.0 μg/kg Q2W	None	15 weeks
20010102 (n = 242)	2	A randomized, open-label study of darbepoetin alfa (novel erythropoiesis stimulating protein, NESP) using fixed and weight-based dosing for the treatment of anemia in subjects with non-myeloid malignancies receiving multicycle chemotherapy	325 μg QW followed by 325 μg Q3W	DA 4.5 μg/kg QW followed by 4.5 μg/kg Q3W	16 weeks
20010162 (n = 81)	2	A randomized, open-label, dose-timing study of darbepoetin alfa administered once every 3 weeks (Q3W) by subcutaneous (SC) injection for treatment of anemia in subjects with non-myeloid malignancies receiving multicycle chemotherapy	6.75 μg/kg Q3W on day 15 of previous chemotherapy cycle	DA 6.75 μg/kg Q3W on day 1 of current cycle	16 weeks
20020132 (n = 2401)	4	A study to assess symptom burden in subjects with nonmyeloid malignancies receiving chemotherapy and Aranesp™	200 μg Q2W	None	24 weeks
20020167 (n = 163)	2	A randomized, open-label, pilot study to evaluate every three week maintenance dosing of darbepoetin alfa therapy in anemic subjects with non-myeloid malignancies receiving chemotherapy	300 μg Q3W	Observation (darbepoetin alfa allowed for low hgb)	22 weeks

Appendix 4A. Amgen-sponsored Studies Not Included in a Combined Analysis: Chemotherapy-induced Anemia

Study Number (n)[a]	Phase	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
20030206 (n = 1493)	4	SYNCHRONICITY: A study to evaluate the effectiveness of Aranesp at 300 mcg Q3W on clinical outcomes in cancer patients with anemia due to chemotherapy	300 μg Q3W	None	13 weeks
20040156 (n = 396)	3b	A randomized open-label study of darbepoetin alfa administered Q3W with or without parenteral iron in anemic subjects with nonmyeloid malignancies receiving chemotherapy	500 μg Q3W	IV iron vs standard practice	16 weeks

[a] Number of subjects randomized who received at least 1 dose of investigational product

Appendix 4B. Amgen-sponsored Studies Not Included in a Combined Analysis: Active Cancer Not Receiving Chemotherapy or Radiotherapy

Study Number (n)[a]	Phase	Study Title	Darbepoetin alfa Starting Dose and Schedule	Control Group	Duration of Treatment
990111A (n = 106)	1/2	An open-label, dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous (SC) injection for the treatment of anemia in subjects with chronic anemia of cancer (Schedule A)	0.5, 1.0, 2.25, 4.5 µg/kg QW	None	12 weeks
990111 B&C (n = 86)	2	A dose-finding study of novel erythropoiesis stimulating protein (NESP) administered by subcutaneous (SC) injection for the treatment of anemia in subjects with chronic anemia of cancer (Schedules B and C)	Schedule B: 6.75 µg/kg Q3W Schedule C: 6.75, 10.0 µg/kg Q4W	Placebo	12 weeks, with optional 12 additional weeks of open-label darbepoetin alfa
20000219 (n = 285)		A randomized, open-label, comparative study to estimate the effect of darbepoetin alfa on hospital days, economic outcomes, and health-related quality of life in subjects with nonmyeloid malignancies and anemia of cancer	3 µg/kg Q2W	Observation for 21 weeks followed by up to 9 weeks of darbepoetin alfa	21 weeks
20030204 (n = 218)	2	A phase 2, randomized, double-blind, placebo-controlled study of darbepoetin alfa administered once every 4 weeks in the treatment of subjects with anemia of cancer	6.75 µg/kg Q4W	Placebo	13 weeks

[a] Number of subjects randomized who received at least 1 dose of investigational product

Appendix 5. Selected Adverse Events in Subjects Enrolled in Clinical Studies Not Included in the Combined Analyses

Study Number		Subjects n	Deaths n (%)	CV/TE Events n (%)	TE Events n (%)
CIA					
980290 A					
all DA arms combined		216	17 (7.9)	26 (12.0)	14 (6.5)
Epoetin alfa TIW		53	6 (11.3)	6 (11.3)	3 (5.7)
20000220		1558	131 (8.4)	182 (24.8)	106 (6.8)
20010102		242	24 (9.9)	57 (23.6)	26 (10.7)
20010162		81	4 (5.9)	7 (8.6)	2 (2.5)
20020132		2401	233 (9.7)	6 (0.2)	1 (<0.1)
20020167					
randomized to DA		99	5 (5.1)	16 (16.2)	10 (10.1)
crossed-over to DA		64	4 (6.3)	5 (7.8)	2 (3.1)
20030206		1493	78 (5.2)	61 (4.1)	33 (2.2)
20040156		396	35 (8.8)	45 (11.4)	24 (6.1)
AOC					
990111A		102	9 (8.8)	7 (6.8)	4 (3.9)
990111B[a]	*DA*	64	1 (1.6)	2 (3.1)	2 (1.6)
	Placebo	22	0 (0)	2 (9.1)	2 (9.1)
20000219[a]	*DA*	226	16 (7.1)	8 (3.5)	0 (0)
	Control	59	3 (5.1)	1 (1.7)	0 (0)
20030204	*DA*	162	11 (6.8)	16 (9.9)	4 (2.5)
	Placebo	56	5 (8.9)	4 (7.1)	0 (0)

Hand-tabulated table based on: *t_aeint_hilev_v2_txgroup_20000220.rtf*
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[a]*Values only from randomized comparative portions of studies; analyzed by randomized treatment group*