Cancer Patient Survival and Erythropoietin

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Abstract
Cancer patients are frequently anemic. Treatment of anemic patients with erythropoiesis-stimulating proteins (ESPs) such as epoetin and darbepoetin is associated with benefits that include a reduced transfusion risk and improved quality of life. The recent reports of two randomized trials in which ESP treatment was associated with a decreased survival raised valid concerns regarding the safety of these agents in oncology practice. Reports of erythropoietin receptors on non-hematologic human tumor cells have increased the level of concern and provided a relatively simple model for the effects of ESPs on tumor progression and resistance to treatment. This article reviews available data, which lead to a number of conclusions: 1) the two trials suggesting a negative impact on survival have serious methodologic issues that may compromise interpretation; 2) when used to treat rather than prevent anemia in cancer patients, ESPs show no significant negative impact on survival outcomes; 3) with the exception of erythroleukemia cell lines, the presence of functional erythropoietin receptors on human tumor cells has not been conclusively shown; and 4) a sound theoretical basis exists, supported by preclinical evidence, that any effect of ESP therapy on tumor outcomes may depend on baseline hemoglobin levels, with different effects when anemic and non-anemic individuals are treated. For the present, it is prudent to withhold ESP therapy unless hemoglobin concentrations fall below 12 g/dL and to titrate treatment to maintain a target of 12 g/dL, with adjustments in therapy to insure that levels do not exceed 13 g/dL. (UNCCN 2005;3:796-804)

“For every problem, there is a solution that is simple, neat, and wrong.”

– H.L. Mencken

Key Words
Erythropoietin, epoetin, darbepoetin, erythropoietin receptor, survival, thrombosis, safety, cancer, hypoxia, fatigue

Theoretical Effects of ESPs on Survival of Cancer Patients

Serious disagreement no longer exists that therapy with erythropoiesis-stimulating proteins (ESPs), including recombinant erythropoietins (EPOs) and darbepoetin alfa, is associated with decreased transfusion requirements and improved functional status and quality of life for anemic cancer patients requiring chemotherapy.1-3 In randomized, placebo-controlled trials in this setting, ESPs have been reported to have an excellent safety profile. However, the recent publication of two randomized studies of ESPs administered to prevent anemia in patients with head and neck cancer undergoing radiotherapy4 or in patients with breast cancer undergoing chemotherapy5 reporting decreased survival associated with ESP therapy raised legitimate concerns regarding safety and forced an important re-examination of available data.

In May 2004, the Oncology Drug Advisory Committee (ODAC) to the Food and Drug Administration (FDA) met to review the data. The briefing documents submitted by the three sponsors of ESPs in use for cancer patients and by the FDA remain a valuable resource and are available on the Internet (http://www.fda.gov/ohrms/dockets/ac/04/briefing/4037b2.htm). These studies also prompted speculation as to the mechanisms by which ESPs may alter survival outcomes for some cancer patients. This article reviews the available data regarding the safety of ESPs administered to cancer patients and defines the substantial limitations of our understanding of the mechanisms involved.
Erythropoietin Receptor 

The EPO Receptor

The effects of EPO on responsive cells are mediated through interaction with the EPO receptor (EPO-R). Several aspects of the biology of this EPO-R are relevant. First, even in EPO-responsive hematopoietic progenitor cells, only a small fraction (2%–5%) of synthesized receptor protein ultimately traffics to the cell surface, a process involving a requisite chaperone, Janus kinase 2 (JAK2). Second, EPO-R turnover is rapid, and these EPO-responsive cells express only very small quantities of functional cell surface EPO-R (approximately 200 receptors per cell). Third, to be functional and initiate signaling on interacting with an ESP, surface EPO-R must be dimerized in a specific conformational structure and associated with JAK. These aspects of EPO-R biology have important implications for attempts to conclusively show the presence of functional EPO-R on non-hematopoietic cells.

Fourth, when expressed on the cell surface in an appropriate configuration and complexed with requisite signal transducing proteins, EPO-R binds endogenous erythropoietin or ESPs and mediates response, including resistance to apoptosis. Because endogenous EPO and ESPs produce the same effects on responsive cells, any satisfactory hypothesis regarding effects of exogenous ESPs on survival, beyond those caused by ambient endogenous EPO, must satisfactorily address this hypothetical quantitative requirement for pharmacologic levels of ligand. Finally, in contrast to instances in which receptor-ligand interactions have been shown to alter the properties of cancer cells, no evidence has been found that EPO-R is an oncogene, and amplification has not been shown in human tumors.

Hemoglobin Concentration and Survival in Cancer Patients

By far the best documented expression of EPO-Rs is on hematopoietic cells, where they mediate effects on the committed erythroid progenitor cells that enhance survival and the production of red blood cells. Hematopoietic cells appear to be the only site of EPO-R expression requisite for viability in mice. Several lines of preclinical evidence suggest that hemoglobin level could impact survival outcomes in cancer.

Anemia is associated with tissue hypoxia, including tumor tissue. The cellular responses to hypoxia include increased expression of key genes, including vascular endothelial growth factor (VEGF), that enhance angiogenesis and resistance to apoptosis. In tumor cells, this hypoxic response may be associated with a more aggressive phenotype and an increased resistance to the effects of radiation and chemotherapy. Using either immunohistochemistry (IHC) on excised human tumors in vitro or insertion of oxygen-sensing probes in vivo, researchers have shown that hypoxia is more common in human cancers when anemia is present (defined in most of these studies as a hemoglobin level of < 11–12 g/dL). In animal models, ESP treatment is associated with improvements in the efficacy of radiotherapy and chemotherapy. Recently, animal model work...
has suggested that ESP treatment may further increase tumor oxygenation independent of effects on hemoglobin. By virtue of its effects on hemoglobin levels and tumor oxygenation, these data predict that ESP therapy for anemic cancer patients would have, if anything, a favorable impact on tumor progression, response to therapy, and survival.

Significantly less preclinical data bearing on the predicted impact of ESP therapy are available outside the setting of anemia (hemoglobin values at baseline > 12–13 g/dL). Increased cellular hypoxia has not been shown in tumors from patients with these hemoglobin levels (compared with truly normal, gender-dependent levels in the 14–15 g/dL range). At these higher hemoglobin levels, the rheologic effects of hematocrit on blood viscosity and flow may predominate, and increased tumor hypoxia may actually occur as hemoglobin rises. Considering the preclinical data, the predicted effect on cancer progression or survival of increasing hemoglobin levels above 12 to 13 g/dL is not at all clear, and adverse effects, including more aggressive tumor behavior and resistance to treatment (because of increased tumor hypoxia), are possible. Clearly, the effects on tumor outcomes mediated through EPO-R on hematopoietic cells could depend on the baseline hemoglobin level.

Vascular Endothelial Cell Activation and Survival in Cancer Patients
The presence of functional EPO-R on normal vascular endothelial cells is relatively well established, however, insufficient data exist regarding their presence on human tumor vasculature to address this issue separately. When ESFs are administered to healthy volunteers, an increase in circulating e-selectin is observed, showing that, despite the presence of normal circulating levels of endogenous erythropoietin at baseline, an increase in endothelial cell activation is associated with pharmacologic levels of ligand. Theoretically, ESPs could adversely impact cancer patient survival through an increase in the risk of fatal thrombosis mediated, in whole or in part, through an effect on vascular endothelium. It seems likely that functional EPO-R is also expressed on endothelial cells in the vasculature of human tumors; if it is, then ESPs could enhance tumor angiogenesis and adversely impact both tumor progression and survival. No direct evidence indicates that the higher concentrations of ligand with ESP therapy compared with endogenous EPO levels promote tumor angiogenesis, but the theoretical possibility exists.

Given the importance of the issue and the relatively strong evidence of EPO-R on normal vascular endothelium, it is surprising that tumor vascular endothelial EPO-R has received so little attention and remains so poorly understood. However, any concern regarding the effects of therapeutic ESPs on tumor angiogenesis in anemic cancer patients must be balanced against the potential for anemia and the resultant tissue hypoxia to increase VEGF expression in tumor cells.

Tumor Cell EPO-R and Survival in Cancer Patients
In recent years, several published papers reported the detection of EPO-R on human cancer cells, using either established cell lines or tumor samples. Given the well-known problems of extrapolating from tumor cell lines to human cancer, the primary tumor data are much more interesting and have attracted more attention. Several methodologic issues must be understood in critically evaluating this literature. First, because human tumors are a mixture of malignant cells and benign stroma (including vascular endothelium) and also contain some blood (including circulating progenitor cells), the most straightforward approach to protein detection—Western blotting—is problematic. Moreover, variations in EPO-R expression within a tumor cannot be characterized using this technique. Similar issues apply to the use of polymerase chain reaction techniques, which in addition are not informative as to whether the gene is actually translated into protein.

Most published studies have therefore used IHC to detect EPO-R in human tumor samples. The limitations of this approach include failure to differentiate between cytosolic and membrane-bound EPO-R protein and an inability to determine whether detected protein represents functional receptor (dimerized and JAK-associated). Both the Western blot and IHC approaches to EPO-R detection on clinical tumor samples have another very important and frequently overlooked limitation: the availability of immuno-reagents that have been rigorously shown to specifically detect EPO-R. Most reported studies used commercially available polyclonal rabbit anti-sera to human EPO-R. Although these reagents clearly bind EPO-R, they equally clearly bind other non–EPO-R proteins. Furthermore, the proteins that have been bound and are therefore assumed to be EPO-R
in non-hematopoietic human cancer cell lines are not EPO-R.41,42

One of the most definitive demonstrations of non-specificity of these commercial reagents is their binding to tissues of EPO-R knockout mouse embryos.42 Until immuno-reagents rigorously shown to be specific for EPO-R are available, it will not be possible to definitively show the presence of EPO-R protein on human tumor cells using IHC or Western blot.

A more specific technique—binding of radiolabeled EPO with determination of EPO-R number and affinity—is feasible for use with cell lines. However, because of the requirement for fresh, viable tumor cells separated from stromal cells using cumbersome micro-dissection approaches, this technique is difficult to use to show EPO-R on cells from human tumor samples. When this technique has been used to detect EPO-R on human cancer cell lines, receptors have been detected on erythroleukemia lines and not on lines derived from non-myeloid malignancies. To date, results of micro-dissection or radiolabel-binding studies have not been reported for human tumor samples. Given the serious limitations in the techniques and studies reported to date, functional EPO-Rs have not been definitively shown to be present on human cancer cells. Any theory that assumes that ESPs act to alter cancer progression and survival through interaction with EPO-R present on the cancer cell must therefore be based on an unproven assumption.

Another approach has been to study the effects of ESPs on in vitro proliferation of human tumor cell lines or the in vivo behavior in xenograft models of tumors derived from these cell lines.43 In the in vitro work, the only consistent observation of an effect of ESPs on tumor cell lines is on erythroleukemia lines. Most reports involving non-myeloid cancer lines have reported no effect, although a few have reported a small increase in proliferative rate when the cell lines are incubated with very high concentrations (1–3 logs greater than peak serum concentrations seen when ESPs are given therapeutically) of ESPs. In the in vivo cell line studies, most have reported either a beneficial effect on tumor biology or response to treatment or no effect. However, a few have reported an effect on tumors that might lead to increased progression or resistance to treatment. These latter studies have taken a very indirect approach, involving the administration of anti-EPO antibodies or soluble EPO-R with an observed increase in apoptotic tumor markers or frank tumor regression, suggesting that endogenous EPO was playing a role in the survival of these tumor cells.45

The implications of these indirect data for the effects of therapeutic ESPs are unclear. If, for instance, endogenous EPO levels are sufficient to maintain an anti-apoptotic phenotype in these tumors, additional therapeutic ESPs may not have any additional effect. Moreover, these data do not provide unambiguous support for the presence of functional EPO-R on these human tumor cell lines because the effects of EPO withdrawal may be mediated, for example, by EPO-R on vascular endothelial cells. Curiously, much of the concern regarding a potential effect of ESPs on the survival of cancer patients has focused on EPO-Rs on tumor cells, despite the absence of data definitively showing their presence on human cancer cells. Potential effects acting through EPO-Rs on hematopoietic cells or on vascular endothelial cells, which are much better characterized, have received much less attention.

When functional EPO-Rs are assumed to exist on human cancer cells, the proposed mechanisms by which exogenous ESPs may alter tumor biology vary. This leads to unclear predictions regarding their effects, if any, on tumor outcomes. One hypothesis assumes that EPO is produced by hypoxic tumor cells and acts, in an autocrine-paracrine fashion, on EPO-R on tumor cells to enhance survival or promote proliferation.40,46 In this model, whether successful treatment of anemia with ESPs would decrease (through decreasing local EPO production) or increase (through enhanced EPO effect on tumor cell ESP-R) tumor progression and resistance to therapy is not clear.

Observed Effects of Anemia and ESPs on Survival Outcomes

As noted previously, several preclinical lines of evidence support conflicting predictions regarding the effects of ESPs on tumor progression, response to treatment, and survival of cancer patients. Several sources of clinical evidence can be reviewed in light of these preclinical predictions.

Epidemiology: Anemia and Survival in Cancer Patients

Observational studies across a very wide spectrum of primary sites have consistently found that anemic patients (usually using hemoglobin “cut points” of 11–12 g/dL) have a diminished survival compared with non-anemic patients.47,48 The obvious weakness of these
data is their observational nature; it is not clear that the two subsets, anemic and non-anemic, differ only in their hemoglobin levels. For some cancers, including cervical and gastrointestinal, anemia may be associated with increased blood loss from larger or more vascular tumors. For other cancers, anemia caused by cytokines elaborated in response to larger or more aggressive cancers may correlate with but not cause an unfavorable outcome. Nevertheless, the consistency of the observation is interesting, especially in light of what is known about the molecular biology of tumor hypoxia, and in harmony with the possibility that anemia might alter cancer biology and that ESP therapy for anemia might positively impact prognosis.

ESP Therapy, Tumor Progression, and Survival in Anemic Cancer Patients

Several randomized, placebo-controlled clinical trials of ESPs for anemic cancer patients receiving chemotherapy have been reported. In most of these studies, tumor progression and survival outcomes have either not been reported or been similar in the ESP and placebo arms. Recently, two randomized, double-blind, placebo-controlled clinical trials in anemic cancer patients have reported a trend suggesting improved progression-free and overall survival in the ESP arms. In a trial of epoetin alfa administered during non–platinum-based chemotherapy for non-myeloid malignancies, a trend suggested improved overall survival in the patients receiving epoetin with either hematologic or non-hematologic (largely breast cancer) malignancy.49 In a trial of darbepoetin alfa given to patients with lung cancer receiving platinum-based chemotherapy, a trend suggesting improved progression-free and overall survival was seen in small cell lung cancer patients receiving darbepoetin.50 It is important to emphasize that survival was not a primary endpoint of either trial, and no measures were taken to assure that the two groups were balanced for baseline prognostic factors. Therefore, the results are hypothesis-generating but far from definitive.

Recently, the Cochrane group published a meta-analysis of randomized trials of epoetin alfa and epoetin beta administered to treat anemic cancer patients during chemotherapy.51-53 The results (Figure 2) show no negative impact on survival in the pooled analysis. Data from later trials of epoetin alpha53 and epoetin beta54 have been published, and similarly show no negative effect on tumor progress or survival. In a briefing document to ODAC and the FDA in May 2004, Amgen provided a similar analysis for the randomized trials of darbepoetin alfa for the treatment of anemic cancer patients receiving chemotherapy (Figure 3). These data were recently published.55 This document reports no negative impact on progression-free or overall survival for darbepoetin alfa. The available evidence supports the conclusion that ESP therapy has no negative impact on survival when used to treat anemia in cancer patients receiving chemotherapy.

ESP Therapy, Tumor Progression, and Survival in Non-Anemic Cancer Patients

To try to determine whether preventing anemia during radiotherapy or chemotherapy enhances survival outcomes during cancer treatment, researchers began several clinical trials in which patients who were not anemic were randomized to receive ESPs or placebo during chemotherapy or radiotherapy. These trials have been specifically designed to study the relationship of ESP therapy to tumor progression or survival endpoints. Recently, the results from two of these trials were reported and suggested that ESP therapy was associated with a reduction in progression-free and overall survival. These two reports have raised concerns regarding the safety of ESPs in oncology practice.
In one trial, patients with cancers of the head and neck undergoing radiotherapy (without adjunctive chemotherapy) were randomized to receive either epoetin beta or placebo during radiotherapy.\textsuperscript{4} Based on an analysis of all randomized subjects, patients receiving epoetin beta were noted to have a decreased duration of both locoregional disease control and survival.

The authors suggest that the effects of epoetin beta may be mediated by EPO-R on tumor cells, conferring a relative resistance to radiotherapy, a position recently buttressed using preclinical data.\textsuperscript{40,56} However, two aspects of this trial made interpretation difficult. First, randomization failed to balance several important prognostic factors: the placebo group included fewer current smokers, fewer tumors that relapsed before treatment, fewer T4 and N2 lesions, and fewer patients with inoperable tumors. All of these factors could favor improved disease-free and overall survival in the placebo group. Second, an inordinately large number of protocol violations were noted, including deviations in radiotherapy, failure to assess local control (the primary endpoint of the trial), and study medication errors. When the analysis is limited to patients who received the correct protocol-specified radiation, underwent primary endpoint assessment, and received the correct study medication, the differences in progression-free and overall survival outcomes in the 2 study groups are eliminated. Even for the intent-to-treat population, the observed difference in local progression and survival is entirely limited to the subset of patients with hypopharyngeal tumors.

One aspect of this trial that merits emphasis is that patients in the epoetin beta group may have had hemoglobin overcorrection to a level associated with tumor oxygenation levels lower than occurred in the placebo group. After 9 weeks, the mean hemoglobin concentration in the epoetin beta group was 15.4 g/dL; in the placebo group, it was 12.9 g/dL. The findings in this trial conflict with a previous non-randomized study of patients with head and neck cancer in which treatment with ESPs to maintain hemoglobin levels greater than 14.5 g/dL appeared to enhance, rather than diminish, the response to radiotherapy.\textsuperscript{57}

In the second study, patients with metastatic breast cancer who were not to have received chemotherapy for their metastatic disease were randomized to receive epoetin alfa or placebo during their initial chemotherapy treatment.\textsuperscript{58} The trial was closed early because of an increased number of deaths in the first 6 months in the epoetin alfa arm compared with the placebo group (41 vs. 16). The most frequent cause of these early deaths as reported by the investigator was tumor progression (13 of 16 in the placebo group and 28 of 41 in the epoetin group) and thromboembolic complications (1 of 16 on placebo; 5 of 41 on epoetin). Paradoxically, however, for the entire patient population (N = 939) no increase was seen in the frequency of tumor progression noted in the epoetin group.

However, ineligible patients with more advanced, refractory disease may have been enrolled in the protocol. Unfortunately, detailed prognostic information (HER-2/neu status, sites of disease) was not collected, and constructing a valid hazards model to determine whether imbalances in baseline prognostic factors between the groups explains the differences in survival is not possible, although the patients receiving epoetin were older, had a lower performance status, and had more thrombotic risk factors.

Several studies of ESPs given to prevent anemia during cancer treatment are ongoing, which should help to address this issue. These include a large trial in patients receiving radiotherapy for head and neck cancer, a trial of epoetin beta for patients with breast cancer receiving chemotherapy, and a trial of darbepoetin alfa for patients with small cell lung cancer. For the present time, the safety of ESPs initiated for the treatment of cancer patients with hemoglobin levels greater than 12 g/dL has not been established and should not be undertaken outside the setting of a randomized clinical trial designed to carefully assess survival.
ESP Therapy and Thrombosis in Cancer Patients

Researchers recognize that ESP therapy increases thrombosis risk in dialysis patients. Proposed mechanisms for this include rapidly rising or high hemoglobin levels, increasing blood pressure and viscosity, activation of vascular endothelium, and activation of platelets by young red blood cells or ESPs and thrombopoietin. Until recently, it was not clear that ESP therapy was associated with an increased incidence of thrombosis in cancer patients, who are already at increased relative risk and who do not have the volume dysregulation and hypertensive tendencies typical of dialysis patients.

More recently, several studies, most often involving patients receiving chemotherapy and radiotherapy and/or with gynecologic malignancies, have reported increased thrombotic complications associated with ESP therapy. Both the Cochrane meta-analysis and the briefing documents submitted by the industry sponsors to ODAC show an increased risk of thrombosis associated with ESP therapy in cancer, with a relative risk of approximately 1.6 or an absolute increase from 3% to 4% to 6%. The mechanism through which ESPs increase thrombosis risk has not been established. No consistent relationship has been found to rate of hemoglobin rise or peak hemoglobin level in the available data. Although some thrombotic complications are potentially life-threatening, the observed and reported incidences of myocardial infarction, cerebrovascular accidents, and pulmonary embolism are clearly not sufficient to explain the diminished survival seen in the two trials that have raised concern. If ESP therapy is associated with a negative impact on survival in some clinical settings, the available data do not support the conclusion that the effect is mediated primarily by fatal thrombotic complications.

Conclusions

The treatment of anemia with ESPs clearly benefits anemic cancer patients, with reductions in red cell transfusion risks and relief of fatigue. When ESPs are used to treat rather than prevent anemia, no current evidence exists that ESPs adversely impact tumor progression, response to treatment, or survival. Recently, reports of two trials of ESPs given to prevent rather than treat anemia raised concerns that ESP therapy may accelerate tumor growth or promote resistance to treatment. However, methodologic concerns regarding these trials suggest that we must await the results of ongoing larger trials before drawing conclusions regarding the safety of preventative use of ESPs. After all, increasing hemoglobin levels to the 12 to 13 g/dL range may possibly be associated with reductions in tumor cell hypoxia and a beneficial effect on response to treatment, and further increases in hemoglobin levels, especially to supra-normal levels, may cause a paradoxical increase in tumor cell hypoxia because of the rheology of blood and the anatomy of tumor vasculature, resulting in relative resistance to treatment.

For the present, it is prudent to withhold starting ESP therapy until the hemoglobin level is less than 12 g/dL and to titrate treatment after it is begun to maintain a target level of 12 g/dL, withholding treatment and subsequently adjusting doses if the hemoglobin level exceeds 13 g/dL. Such an approach represents a responsible response to the existing data.

References


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