rHuEPO and Improved Treatment Outcomes: Potential Modes of Action

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ABSTRACT
Within the past decade, clinical trials have shown that the presence of anemia can diminish the physical status, functional abilities, and overall quality of life (QOL) of cancer patients and can negatively influence the outcome of their treatment. However, recent preclinical and clinical studies have also shown that increasing hemoglobin levels by administering recombinant human erythropoietin (rHuEPO, epoetin alfa) may ameliorate anemia and, in doing so, improve QOL and possibly result in better treatment outcomes following radiotherapy, chemotherapy, or a combination of these modalities. Several mechanisms by which rHuEPO may improve treatment outcome have been proposed, including correction of tumor hypoxia, increased sensitivity of tumor cells to radiotherapy and chemotherapy, correction of anemia and its associated symptoms (particularly fatigue), and immune-modulated effects of rHuEPO on tumor growth. Improvement of tumor oxygenation by rHuEPO could affect treatment outcome in two ways. First, correction of hypoxia results in the downregulation of hypoxia-inducible factor 1 (HIF-1), a key regulator of cellular adaptive responses to hypoxia (e.g., angiogenesis), including many pathways that are important for tumor growth and metastasis. Interruption of the HIF-1 pathway not only limits growth of the primary tumor but also reduces the potential for the development of more aggressive tumors and metastatic spread, which could ultimately improve treatment outcome. Second, within the tumor, it is the hypoxic cells that are resistant to oxygen-dependent radiotherapy and chemotherapy, and improvement in their oxygenation would increase their sensitivity to the cytotoxic effects of such treatment.

Correction of anemia and its associated symptoms, particularly fatigue, can have a beneficial effect on patient QOL, and this in turn may translate into greater tolerance of radiotherapy and chemotherapy, allowing patients to receive full doses and on-schedule dosing.

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:

1. Relate hypoxia to gene expression.
2. Relate hypoxia to angiogenesis.
3. Discuss the possible relationship between tissue hypoxia and metastatic potential.

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and thus have an increased likelihood of a therapeutic response. Lastly, results of a study using a murine model of multiple myeloma have indicated that rHuEPO may induce an immune-mediated antitumor effect. Therefore, additional research is warranted to further explore the biologic actions of rHuEPO and to determine their relevance to therapeutic outcome. The Oncologist 2004;9(suppl 5):41-47

INTRODUCTION

Anemia is a common complication of cancer and cancer treatment, occurring in more than 50% of patients [1]. Cancer-related anemia is associated with a broad range of symptoms (e.g., fatigue, dizziness, palpitations, dyspnea, impaired cognitive function) that can adversely affect a patient’s functional capacity and mental status and diminish their quality of life (QOL) [2]. Moreover, results of recent studies suggest that anemia may negatively influence locoregional control and overall survival in patients undergoing radiation therapy or radiochemotherapy [3-9] and may be related to poorer outcome in patients receiving chemotherapy [10]. Additionally, studies have shown that administration of recombinant human erythropoietin (rHuEPO, epoetin alfa) to anemic cancer patients may elevate their hemoglobin (Hb) levels, thereby ameliorating anemia and improving QOL; rHuEPO administration may also improve treatment-related outcomes [11-16]. Several mechanisms by which rHuEPO may improve cancer therapy outcomes have been proposed, including correcting tumor hypoxia, increasing the sensitivity of tumor cells to radiotherapy and chemotherapy, improving QOL, and having a direct or immune-modulated effect on tumor cells. The diversity of these proposed mechanisms reflects a growing body of evidence suggesting that erythropoietin has biological activities in addition to those related to its well-established hematopoietic effects [17].

CORRECTION OF TUMOR HYPOXIA

Hypoxia, a characteristic feature of locally advanced solid tumors, is now recognized as an important factor in promoting tumor resistance to radiotherapy, chemoradiotherapy, and some chemotherapeutic agents [10, 18]. Treatment resistance may develop directly as a result of deprivation of the molecular oxygen required for these agents to exert their therapeutic activity. This effect may also develop indirectly, as a result of hypoxia-mediated alterations in gene expression and changes in the tumor cell proteome and genome that lead, in turn, to adaptive responses that help tumor cells survive (Fig. 1) [19, 20].

Relationship Between Hypoxia and Anemia

Results of both experimental and clinical studies have provided evidence of a possible relationship between anemia and tumor hypoxia. Lavey showed a strong correlation \( r = 0.57; p < 0.001 \) between increased hematocrit and increases in tumor oxygenation in mice with radiation-induced chronic renal failure [21, 22], and Kelleher et al., using a rat model, showed that the oxygenation of tumors <1.4 ml was lower in anemic than in nonanemic animals, a condition the investigators were able to partially correct with administration of rHuEPO [23, 24]. In the clinical setting, a relationship between anemia and tumor hypoxia was demonstrated by Vapen et al., who found that oxygen tension (pO2) values were significantly \( (p = 0.05) \) lower in cervical cancer patients with severe anemia (Hb ≤11.0 g/dl) than in those without anemia (Hb >14.0 g/dl) [25], and that even mild anemia in patients with breast cancer was a major causative factor for the development of hypoxia or anoxia [26]. Similarly, Becker et al. found a significant \( (p = 0.0001) \) association between severe anemia and poor tumor oxygenation in patients with head and neck squamous cell carcinomas [27]. Data from a clinical study that examined radiation-induced changes in pO2 in cervical cancer patients showed that a baseline Hb level <13 g/dl, compared with an Hb level ≥13 g/dl, was associated with significantly poorer tumor oxygenation (median pO2 12.4 mmHg versus 28.1 mmHg; \( p = 0.003 \)) and a higher rate of treatment failure at 1 year (56% versus 22%; \( p = 0.046 \)) [28], further supporting the theory of a direct relationship between a lower Hb level and a high level of hypoxia [29].

Hypoxic Gene Control and Angiogenesis

The relationship between anemia and tumor hypoxia, coupled with the demonstrated role of tumor hypoxia in malignant progression and treatment resistance, suggests that

![Figure 1. Impact of anemia on long-term outcome in patients undergoing radiotherapy, chemotherapy, or a combination of these modalities. Adapted with permission from Dunst [71].](image-url)
administration of rHuEPO may improve therapeutic outcome by correcting anemia, thereby decreasing tumor hypoxia. At the molecular level, a decrease in tumor hypoxia would result in the downregulation of hypoxia-inducible factor (HIF)-1α, the active moiety of HIF-1. Under hypoxic conditions, HIF-1 activates the transcription of genes that encode erythropoietin, vascular endothelial growth factor (VEGF), and many other survival and growth-regulating factors that act to compensate for the detrimental effects of low oxygen [30, 31]. In experimental studies, exposure of human melanoma cells to hypoxia resulted in enhanced secretion of VEGF, increased stimulation of angiogenesis following intradermal inoculation in BALB/c-nu/nu mice, and increased lung colonization potential following i.v. inoculation in BALB/c-nu/nu mice [32, 33]. As shown in Figure 2, the metastatic efficiency of human melanoma cells increased with increasing duration of exposure to hypoxia [34].

Based on the observations of upregulation of HIF-1 in tumor cells, upregulation of VEGF by HIF-1, and VEGF promotion of tumor growth, it seems logical that interruption of the HIF pathway would decrease angiogenesis and impede tumor growth, a hypothesis supported by the findings of both in vitro and in vivo studies [35-39]. As an extension of this logic, it is conceivable that correction of anemia by administration of rHuEPO would decrease accumulation of HIF-1α and/or upregulation of HIF-1, which potentially would lead to reductions in VEGF secretion, angiogenesis, and the VEGF-enhanced potential for increased tumor growth and aggressiveness. In turn, tumors without these adaptive strategies should be more responsive to anticancer therapy, resulting in better therapeutic outcomes.

**Figure 2.** Human melanoma cells were exposed to hypoxia in vitro, reoxygenated, and, less than 1 hour later, were inoculated i.v. into BALB/c-nu/nu mice for formation of lung colonies. The plot shows the number of lung colonies per $1 \times 10^5$ viable cells versus time under hypoxic conditions. Adapted with permission from Rofstad et al. [33, 34].

**IMPROVED SENSITIVITY TO RADIOTHERAPY AND CHEMOTHERAPY**

The findings of several preclinical and clinical studies suggest that correction of anemia by administration of rHuEPO may increase the sensitivity of tumor cells to radiation therapy and chemotherapy [40-46]. In a study that used a tumor-associated and chemotherapy-induced anemia model, prevention of anemia by rHuEPO resulted in a significant enhancement of the efficacy of radiation therapy in inhibiting tumor growth [44, 45]. Specifically, growth delays of 9.5 days were noted in rHuEPO-treated anemic animals, compared with 4.5 days in untreated animals and 12.0 days in nonanemic controls. Similar results were obtained in a study that employed a carboplatin-induced anemia model [43, 47]. Results of a small group of clinical studies also suggest that using rHuEPO to correct anemia improves sensitivity to radiation therapy, as evidenced by increases in locoregional tumor control following radiation therapy and chemoradiotherapy [15, 48, 49], although a recent double-blind, randomized study has shown conflicting results [50]. In that study, patients who received epoetin beta had significantly (p = 0.0008) poorer locoregional progression-free survival than those who received placebo.

Interim analyses of data from a recent multicenter, randomized study suggest that administration of epoetin alfa in combination with chemoradiotherapy may also provide a survival benefit [51]. In that study, patients with high-risk cervical cancer who received sequential adjuvant chemoradiotherapy plus epoetin alfa showed a trend toward a longer relapse-free survival than patients who received chemoradiotherapy only. The proportions of patients remaining recurrence free after a median observation time of 64.5 weeks were 89% for epoetin-alfa-treated patients versus 78% for patients not treated with epoetin alfa (p = 0.04; log-rank test, one-sided). In those four studies, the improvements in sensitivity to treatment may be due to better oxygenation of the tumor subsequent to an increase in Hb level. Because the cytotoxic activity of radiation therapy and some chemotherapeutic agents is oxygen dependent, rHuEPO, by increasing the levels of intracellular oxygen, may enhance the sensitivity of target cells to radiation- and chemotherapy-induced damage and ultimate cell death; this, in turn, could lead to improved therapeutic outcomes. Results of several preclinical and clinical studies of rHuEPO and its effects on treatment outcome are discussed elsewhere in this supplement [52, 53].

**CORRECTION OF ANEMIA- AND FATIGUE-RELATED INTOLERANCE OF CANCER THERAPY**

Another means by which rHuEPO may improve treatment outcomes is its beneficial effects on a patient’s physical state and overall QOL. As indicated earlier, cancer-related
anemia is associated with an array of symptoms that can decrease patients’ functional abilities and diminish their QOL. Both double-blind, placebo-controlled clinical trials and open-label, single-arm, community-based studies have demonstrated the efficacy of epoetin alfa in increasing Hb and hematocrit levels, thereby ameliorating anemia and reducing transfusion requirements in patients with cancer-related anemia [11, 13, 14, 16, 54-56]. Four of the more recent studies evaluated the effects of epoetin alfa on QOL using the Cancer Linear Analog Scale (CLAS, also known as the Linear Analog Scale Assessment or LASA) [11, 13, 14, 16] and the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale [13, 14, 16]. The results of all four studies demonstrated significant (range \( p < 0.01 \) to \( p < 0.001 \)) improvements in QOL measures that were related to an epoetin alfa-stimulated increase in Hb level. Of particular interest was the finding in the double-blind, placebo-controlled trial of a significant difference favoring epoetin alfa over placebo for the FACT-An Fatigue subscale (\( p = 0.0040 \)) (Fig. 3) [16]. Importantly, the results of the univariate analysis of the QOL data from the latter study [16] were confirmed by a multiple linear regression analysis. Results of the regression analysis showed a significant (\( p < 0.05 \)) advantage of epoetin alfa over placebo for each of the five primary cancer-sensitive, Hb-sensitive scales used (CLAS Energy Level, Ability to Do Daily Activities, and Overall QOL scales, FACT-General [FACT-G] Total scale, and FACT-An Fatigue subscale) after adjustment for disease progression and other possible confounding variables [57]. In a related analysis, Cella et al. conducted an internet survey to establish normative data for FACT-An QOL scales and subscales that could be used to determine the clinical relevance of QOL data obtained in clinical trials [58]. Comparison of these normative data with the QOL data obtained in the previously mentioned placebo-controlled study [16] demonstrated that treatment with epoetin alfa led to clinically meaningful improvements in QOL in anemic cancer patients.

Fatigue is now the most commonly reported symptom in cancer patients and, of all anemia-related symptoms, appears to exert the greatest negative impact on QOL [59]. In a survey of 397 cancer patients, 91% of the 301 patients who reported fatigue stated that it prevented them from leading a normal life, and 88% indicated that it forced them to alter their daily routines [60]. Nevertheless, fatigue in cancer patients is not often discussed and infrequently treated [61]. From the clinical perspective, the symptoms of anemia, particularly fatigue, are a concern because they can compromise the effectiveness of treatment by decreasing a patient’s willingness to endure a full course of therapy and also because they can negate the benefits of any gains in survival time by causing a severe and prolonged diminution in QOL [10]. Thus, rHuEPO therapy may improve treatment outcome by preventing or ameliorating anemia and its associated symptoms, thereby potentially increasing a patient’s ability to tolerate various forms of therapy and receive them at the correct dose and in a timely manner. Further, rHuEPO may improve outcome via other effects that ultimately result in improvement in a patient’s sense of well-being and overall QOL. For example, cognitive problems (e.g., memory loss, decreased concentration, impaired

![Figure 3. Effects of epoetin alfa on QOL as determined in a randomized, double-blind, placebo-controlled trial in patients receiving nonplatinum chemotherapy. Comparison of mean change scores are shown for: A) the CLAS (comprising Energy Level, Ability to Do Daily Activities, and Overall QOL) and B) the FACT-An (comprising FACT-G, Fatigue subscale, and Anemia subscale) QOL instruments. *Indicates \( p \) value for the Anemia subscale is unadjusted; all other \( p \) values are adjusted for multiple comparisons. Reprinted with permission from Littlewood et al. [16].](image-url)
language skills) [62, 63] frequently occur in patients receiving chemotherapy, particularly those who have undergone high-dose chemotherapy for breast cancer. Results of ongoing trials suggest that treatment with epoetin alfa may be of value in improving cognitive dysfunction in these patients [64]. A potential mechanism of action for this effect is based primarily on studies in animal models demonstrating that rHuEPO can protect neurons from ischemic insult [65]. These encouraging results have also spurred investigation of the potential therapeutic benefits of epoetin alfa in patients who have had ischemic strokes [66].

Emerging data suggest that rHuEPO may additionally suppress pulmonary toxicity induced by bleomycin (Blenoxane®; Bristol-Myers Squibb; Princeton, NJ) and subsequently improve lung function. In this instance, rHuEPO appears to improve outcome by inhibiting bleomycin-induced activation of capillary endothelium, thus preventing its destruction [67, 68]. This effect has the added potential for improving the patient’s QOL.

**DIRECT OR IMMUNE-MODULATED EFFECTS**

Approximately 60%-90% of patients with multiple myeloma experience anemia, which, in these patients, is caused mainly by inadequate erythropoietin production [69]. In a clinical trial in multiple myeloma patients, Mittelman et al. found that several patients had lived longer than expected and that their disease had stabilized [70]. As epoetin alfa was the only therapy administered during most of the follow-up period, the investigators hypothesized that rHuEPO was exerting a previously unrecognized biologic effect on the disease. To test this hypothesis, the investigators examined the effect of epoetin alfa on tumor progression and the immune system using the MOPC-315 murine myeloma model. Ludwig [52] describes the specifics of that study in detail. Briefly, Mittelman et al. found that administration of epoetin alfa to MOPC-315 tumor-bearing BALB/c mice resulted in complete tumor regression in up to 60% of the mice treated with epoetin alfa [70]. On rechallenge of regressor mice with additional MOPC-315 tumor cells, no new tumors developed, indicating that epoetin alfa can elicit an effective immune response. Additional testing showed that the epoetin alfa-triggered regression was tumor specific, with memory of tumor antigen and, further, that this response was T-cell mediated.

**SUMMARY**

By depriving tumors of oxygen, anemia may diminish the efficacy of oxygen-dependent radiotherapy and chemotherapy, thereby adversely affecting patient outcome. Additionally, anemia may worsen patient outcome by promoting tumor hypoxia. This leads in turn to induction of HIF-1, which mediates cellular responses that enable the tumor cells to survive, propagate, and spread in the hypoxic milieu. The results of preclinical and clinical studies suggest that correction of anemia with rHuEPO may improve patient outcome by increasing the sensitivity of tumor cells to the cytotoxic actions of radiotherapy and chemotherapy, and by forestalling induction of HIF-1 pathways. Other mechanisms by which rHuEPO may improve patient outcome are also emerging, as previously unsuspected biologic actions of this agent are being discovered. For example, recent research has indicated that rHuEPO may have an antitumor effect mediated by T-cells and may act in the central nervous system to ameliorate chemotherapy-related cognitive dysfunction and provide protection against ischemic insult. Clearly, additional research is needed to develop further knowledge and understanding of the role of rHuEPO in improving treatment outcomes.

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