

Review

Preclinical and Clinical Effects of Erythropoietin in the Management of Anaemia in Patients with Non-small Cell Lung Cancer

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Abstract. *The myelosuppressive toxicities of chemotherapy are one of the principle reasons for the overall failure of some agents to have a meaningful impact on responses and survival in cancer, and anaemia is a common side-effect of almost all cytostatic drugs used clinically. As regulators of haematopoietic homeostasis, cytokines mediate cellular proliferation, differentiation and survival. Among the various growth factors currently available, erythropoietin (EPO) is the principle factor responsible for the regulation of red blood cell production during steady-state conditions and for accelerating recovery following cytostatic bone marrow depletion. Many studies have provided evidence that EPO is able to correct and to prevent anaemia in approximately 64% of cancer patients with subsequent reduction of blood transfusion requirement. Among the prognostic factors for survival in patients with advanced non-small cell lung cancers (NSCLC), anaemia is associated with reduced response rates and quality of life, and a poorer prognosis. Recently, some studies suggest a possible relationship between increased haemoglobin levels and survival in NSCLC patients. Furthermore, there is evidence that NSCLC patients with high haemoglobin levels have a better outcome after radio- or chemotherapy. Although the highest rate of transfusion-dependent patients (34%) has been observed in patients suffering from NSCLC, there are no universally accepted guidelines addressing the most effective methods of monitoring NSCLC patients for anaemia. Thus,*

further randomized, controlled trials are needed to evaluate the effect of any therapeutic intervention against anaemia on survival and disease control in patients with NSCLC.

Lung cancer is the most frequent cancer worldwide. In the United States it accounts for almost one third of all cancer deaths in males and 25% in females (1). Non-small cell lung cancers (NSCLCs) comprise about 80% of all cases. The majority of patients present with, or subsequently relapse with advanced stage disease. Due to the high metastatic potential of NSCLC, the widespread organ involvement can cause a great variety of symptoms. Local growth and systemic effects (malaise, anorexia, anaemia, asthma and fever), however, are the dominating symptoms (2). The outcome of untreated patients with advanced NSCLC is predictable with a median survival time of four months, and a 1-year survival rate of 10% to 15% (3). Chemotherapy has demonstrated benefits for patients in terms of prolongation of disease-free survival and improvement in quality of life (4, 5). The 1-year survival of patients with advanced and metastatic NSCLC has gradually increased in the last two decades to approximately 40-50%, not least due to the use of novel chemotherapeutic agents in selected patient groups (6, 7). In 2003, the American Society of Clinical Oncology updated their clinical practice guidelines for the treatment of unresectable NSCLC (8). Combination chemotherapy with cisplatin and a new agent such as vinorelbine, paclitaxel or gemcitabine was recommended as the standard of care for advanced NSCLC patients, however, the best platinum-based doublet regimen was not defined. Recently, Schiller *et al.* (9) published a randomized phase III study of four chemotherapy regimens in NSCLC. When compared with the reference regimen of cisplatin/paclitaxel none of the three investigational arms demonstrated superior efficacy in terms of response rates, progression free and overall survival (Table I), suggesting that all four regimens are valid options for treatment of NSCLC. Regardless of the treatment selected, grade 3-4 anaemia occurred in many

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Table I. Comparison of four chemotherapy regimens used for treatment of NSCLC patients.

Regimen	Response rate (%)	Time-to-progression (months)	Median survival (months)	1-Year survival (%)	Grade 3-4 anaemia and thrombocytopenia (%)
Cisplatin, paclitaxel	21	3.5	7.8	31	18
Cisplatin, gemcitabine	21	4.5	8.1	36	77
Cisplatin, docetaxel	17	3.6	7.4	31	19
Carboplatin, paclitaxel	15	3.3	8.2	35	20

patients. Performance status is the single most important prognostic factor in advanced NSCLC (Table II). Patients with an ECOG (Eastern Cooperative Oncology Group) performance status of 0 and 1 are more likely to tolerate combination chemotherapy better and have a prolonged overall survival compared to those with ECOG performance status equal or greater than 2 (10). Among the prognostic factors for survival in patients with advanced NSCLC, anaemia is a frequent complication of cancer therapies and is associated with reduced response rates and decreased quality of life (11). Since some studies suggest a possible relationship between increased haemoglobin levels and survival in lung cancer patients (12, 13), the aim of this paper is to assess the role of erythropoietin (EPO) in the treatment of NSCLC.

Mechanism of Action of EPO

In humans, hypoxia induces increased EPO expression stimulated by a DNA-binding protein, hypoxia-induced factor-1 (HIF-1). EPO is then secreted into the plasma and after arriving in the bone marrow it binds to EPO receptors (EPO-Rs) on the surface of erythroid progenitor cells. The binding triggers a conformational change that brings EPO-R-associated Janus family tyrosine kinase 2 (JAK2) molecules into close proximity, stimulating their activation by transphosphorylation (14-16). Subsequently, JAK2 molecules phosphorylate eight tyrosine residues in the cytoplasmic domain of the EPO-R, which then serve as docking sites for various Src homology 2 (SH2) domain-containing intracellular signaling proteins (17). These proteins, in turn, are tyrosine phosphorylated and activated. One of these proteins is a signal transducer and activator of transcription (STAT5) that, on phosphorylation by JAK2, dissociates from the EPO-R, dimerizes and then translocates to the nucleus to activate numerous target genes, including the apoptosis inhibitor bcl-x (18) (Figure 1). The inhibition of apoptosis by the EPO-activated JAK2/STAT5/bcl-x pathway is important for erythroid differentiation. JAK2 deficiency causes embryonic death due to the absence of definitive erythropoiesis (19). Furthermore, mice deficient in STAT5a/5b have anaemia that correlates with the decreased expression of bcl-x and

Table II. Prognostic factors for survival in patients with advanced NSCLC.

Favorable
ECOG performance status 0-1
Single metastasis
Weight loss less than 5 kg
Normal LDH
Normal alkaline phosphatase
Haemoglobin >11 g/dl
Unfavorable
ECOG performance status ≥2
Disseminated disease
Haemoglobin <11 g/dl
Elevated calcium levels
Age >60 years old
Weight loss >5 kg

increased apoptosis in early erythroblasts (20). In addition, full bcl-x knockout mice died in embryogenesis with extensive apoptosis of immature haematopoietic cells, and conditional haematopoietic-specific bcl-x knockout mice had severe anaemia. In both models, bcl-x was required for survival of erythroid cells during terminal maturation (21). A recent study also demonstrated that enforced bcl-x expression can rescue maturation of EPO-deprived erythroid progenitors *in vitro*, suggesting that the major erythropoietic function of EPO is to prevent apoptosis and that *bcl-x* is a critical effector gene (20). However, it is important to note that EPO and EPO-R null mice exhibit a more severe erythropoietic defect than that seen in *bcl-x* null animals, indicating that EPO fosters erythropoiesis through additional effectors. Moreover, data indicate that STAT5-independent mechanisms for *bcl-x* induction also exist (21). In addition to STAT5, EPO induces JAK2-mediated tyrosine phosphorylation and activation of several other intracellular proteins (14) including Shc, which, in turn, may activate: the signaling pathway involved in erythroid cell proliferation, phosphatidylinositol 3-kinase (PI3-K), which may promote survival of erythroid cells, and phospholipase Cγ, which may play a role in erythroid cell proliferation (21).

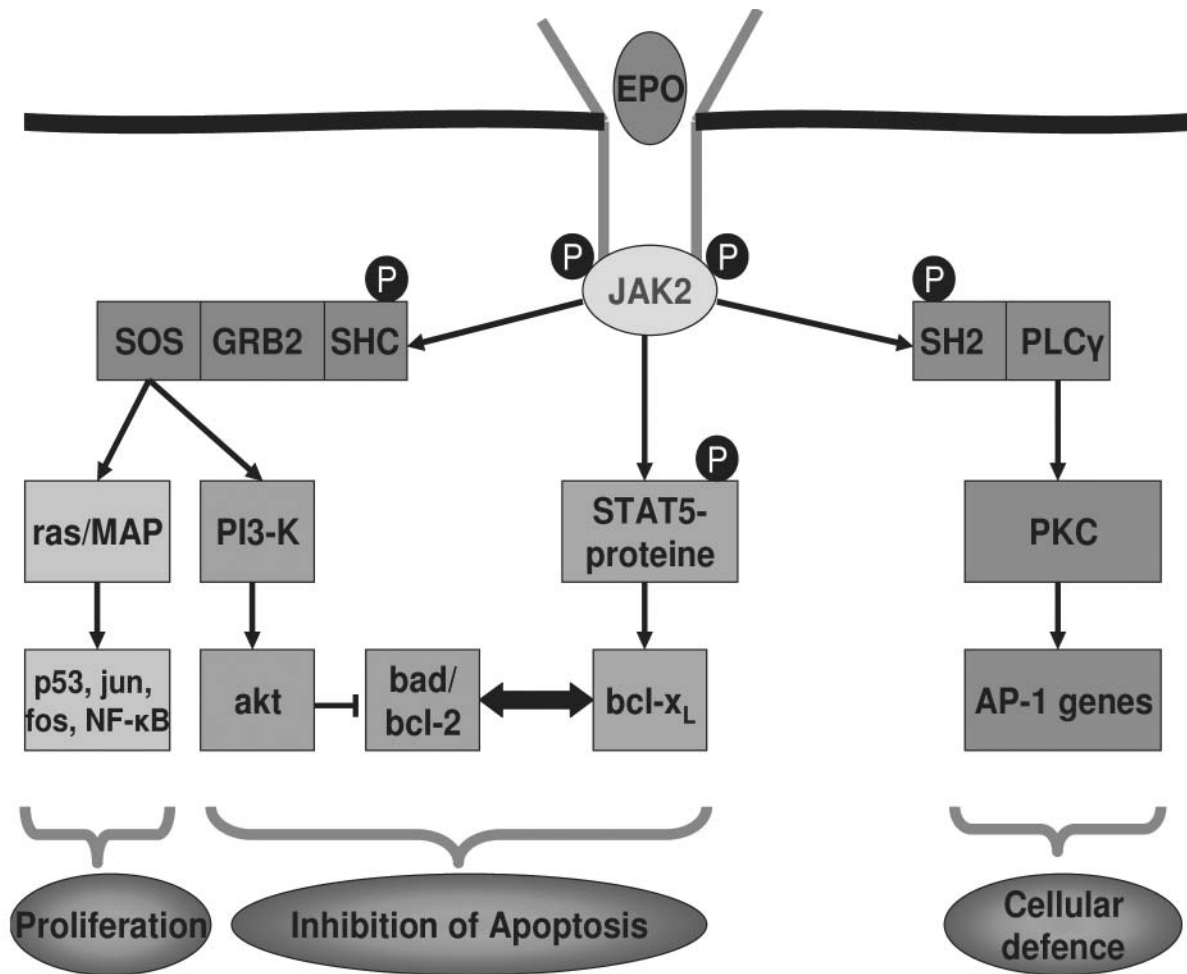


Figure 1. JAK2/STAT5/bcl-x signal transduction pathway of the EPO receptor. P: phosphate.

Cancer-related Anaemia: Molecular Biology

As regulators of haematopoietic homeostasis, cytokines modulate cellular proliferation, differentiation and survival. Specific cytokines influence haematopoietic precursor cell survival by either inhibiting or promoting apoptotic cell death. In addition, cytokines may also have a direct effect on cell proliferation or differentiation. The predominant effect of a specific cytokine on haematopoietic precursor cell fate may depend on the maturation status of the haematopoietic target cell, the lineage commitment of the precursor cell, the concurrent exposure of the precursor cell to other cytokines, or the cellular expression of survival gene products (22). Among the various growth factors currently available, EPO was the first to be used clinically. EPO is the principal factor responsible for the regulation of red blood cell production during steady-state conditions and for accelerating recovery of red blood cell mass following haemorrhage (23).

Although anaemia is frequently associated with malignancy, there are numerous factors that could cause anaemia in cancer patients. In a considerable number of patients, however, there are no causes that are able to explain the anaemia other than the malignant disease itself. Such cancer-related anaemias (CRAs) may have haematological and biochemical similarities to anaemias that occur in other chronic diseases (ACDs). In most cases, CRA is a hyporegenerative, normocytic, and normochromic anaemia characterized by a reduced serum iron and transferrin saturation despite a normal or elevated ferritin level. Recent investigations suggest that CRA is the result of a multifactorial process triggered by the activation of the immune and inflammatory system, and that certain cytokines such as interferon- γ (IFN- γ), tumor necrosis factor α (TNF- α), or interleukin-1 (IL-1) may be involved in the development of CRA. Serum concentrations of these cytokines have been found to be higher in patients with cancer or other chronic diseases (reviewed in (24)). Several

mechanisms have been implicated in the development of CRAs including (i) shortened red blood cell survival, (ii) impaired iron utilization, (iii) suppression of erythroid progenitor cells, and (iv) inappropriate EPO production. In patients with CRA, red blood cells (RBCs) usually survive 60-90 days, compared with 120-day RBC survival in healthy persons (25). There are clinical and experimental data indicating that this effect may be mediated by IL-1 and TNF- α (24). In addition, TNF- α is able to induce dyserythropoiesis and erythrophagocytosis in experimental model systems. Recently, a protein called anaemia-inducing substance (AIS) has been identified in the plasma of patients with advanced cancer (26). AIS decreases the osmotic resistance of RBCs and has also been found to be present in both the cytosol and nuclear fraction of neoplastic cells. The underlying mechanisms of the increased osmotic fragility of RBCs induced by AIS seems to depend on the inhibition of the glucose metabolism (glucose influx, pyruvate kinase activity, and ATP concentration) of these cells. AIS appears to be specific for malignant diseases and not to be associated with chronic inflammatory disorders (26).

In patients with CRAs, an inverse correlation between haemoglobin and ferritin and markers of cellular immune activation (IFN- γ , neopterin) can be found, indicating that activated macrophages may be involved in the alteration of iron metabolism and the development of CRA. Recent investigations have shown that human mononuclear phagocytes down-regulated their transferrin receptor (TFR) expression and ferritin content when exposed to IFN- γ (27). The reduction in iron incorporation, however, is quantitatively lower than the reduction in TFR expression and ferritin content. As a result, ferritin remaining in these cells is approximately three times more saturated with incorporated iron than ferritin in non-activated cells. Moreover, ferritin in IFN- γ -activated monocytes appears to be of the high-iron type, a type of ferritin that takes up iron rapidly and releases it slowly relative to the low-iron type (27, 28).

Another cytokine that may also be involved in the changes in iron metabolism is IL-1. This cytokine is able to increase the production of ferritin, which could act as a trap for iron that might otherwise be available for erythropoiesis (29). Furthermore, in the course of infections, malignancies, and immunological disorders, IL-1, IL-6, and TNF increase the concentration of the acute-phase protein α 1-antitrypsin that is able to inhibit erythropoiesis by impairing transferrin binding to TFR and subsequent internalization of the TFR-transferrin complex (30). IFN- γ , IL-1, and TNF have been reported to inhibit erythropoiesis *in vitro* as well as *in vivo*, and all three cytokines have been shown to suppress erythropoiesis synergistically or to enhance one another's expression (31, 32). Moreover, data from *in vivo* studies suggest that the therapeutic effect of EPO in patients with CRA may in part be due to an overcoming of suppressive

effects of these cytokines on erythroid progenitor cells (32). In patients with CRA, erythroid progenitor cells respond normally to EPO, but EPO response to anaemia appears to be disturbed. The impaired EPO response seen in CRA may be a result of suppressive effects of IL-1 (α or β) or TNF on EPO-producing cells since *in vitro* studies have indicated that these cytokines are able to inhibit the production of EPO in human hepatoblastoma cell cultures and in isolated rat kidneys (33, 34).

The various potential mechanisms underlying CRAs are outlined in Figure 2. To date several experimental and clinical studies indicate that in patients with CRA pharmacological dosages of EPO may not only correct the relative EPO deficiency, but also overcome the suppression of erythroid progenitor cells and the impairment of iron mobilization. Based on these findings, the use of EPO in cancer chemotherapy has increased dramatically over the last few years, as the association between improvement in haemoglobin levels and improvement in quality of life, response rates, and a reduction in fatigue has been recognized (12).

Significance of Hypoxia for Tumour Therapy

Tumor hypoxia has been considered to be a therapeutic problem as tumor tissue oxygen depletion makes solid tumors resistant to ionizing radiation and chemotherapy. Hypoxia may also modulate the proliferation kinetics, the cell-cycle position and the number of tumor cell accumulation in G₀ phase. These changes can, in turn, greatly influence the number of cells destroyed by radiation and/or chemotherapy. Moreover, recent clinical studies suggest that hypoxia enhances malignant progression and increases aggressiveness by clonal selection and genome changes (35). As a result, enhanced metastasis and increased locoregional spread can further increase resistance to therapy. Tumor hypoxia may represent a severe problem for radiation therapy of many cancers because radiosensitivity rapidly decreases if the oxygen partial pressure in a tumor is less than 25-30 mm Hg. Hypoxia-associated resistance to ionizing radiation is multifactorial. The presence of molecular oxygen either increases DNA damage by the formation of (oxygen-derived) free radicals, which occurs primarily after the interaction of radiation with intracellular water, or can prevent repair of DNA damage ("damage fixation") (36). Further evidence suggests that hypoxia-induced proteomic and genomic changes may also have a substantial impact on radioresistance by increasing the levels of certain heat-shock proteins or by increasing the number of cells in a tumor with diminished apoptotic potential or increased proliferation potential of selected clones, both of which have been linked to radioresistance (Figure 3).

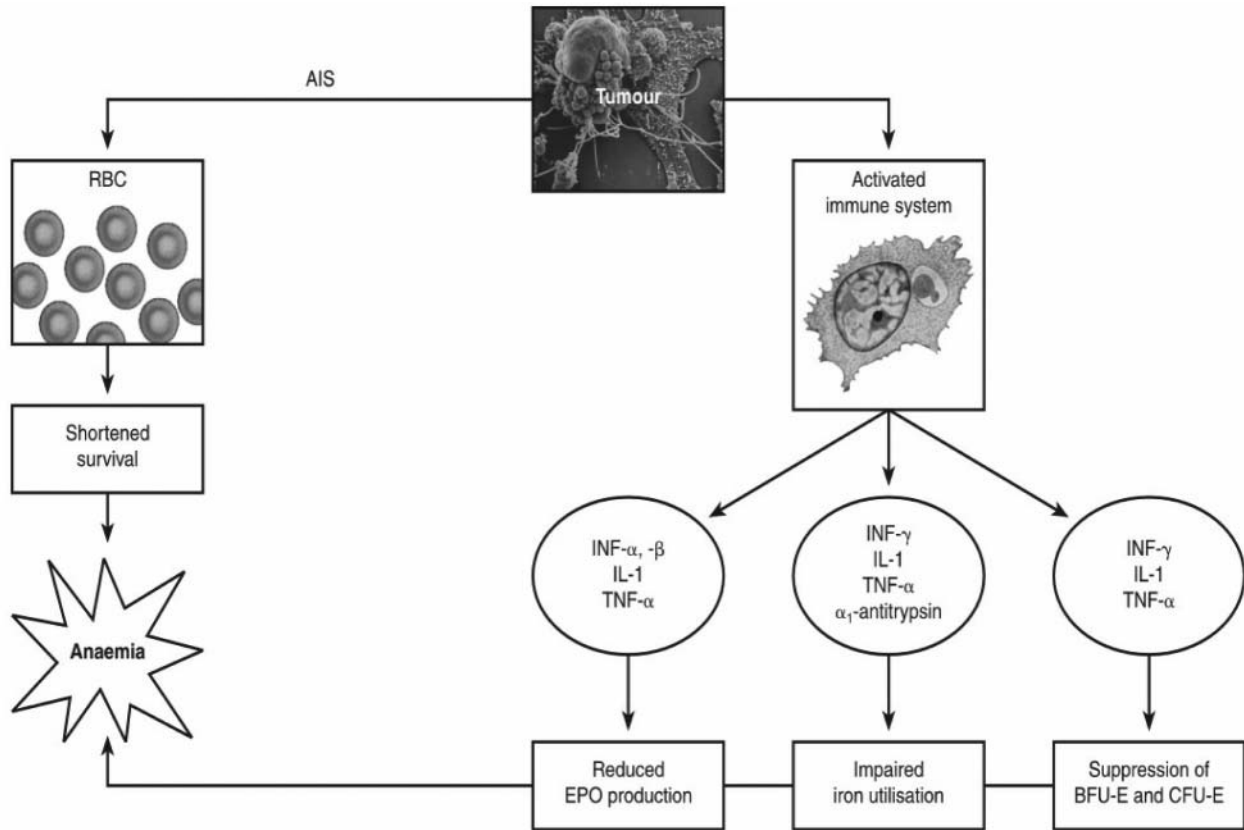


Figure 2. Pathogenic mechanisms involved in the development of cancer-related anaemias. AIS: anaemia-induced substance, IFN: interferon, TNF: tumour necrosis factor, IL: interleukin, BFU-E: burst-forming unit-erythroid, CFU-E: colony-forming unit-erythroid.

The first clinical observation of the haemoglobin effect in radiotherapy came from a study of patients with carcinoma of the uterine cervix (37). This effect has subsequently been confirmed by numerous clinical studies reporting an impaired radiocurability of anaemic patients (35), most probably the result of hypoxia-related radioresistance; a significant influence of haemoglobin levels on the outcome of radiotherapy has been convincingly documented for a panel of human carcinomas including lung cancer patients. First clinical trial experiences using EPO to prevent or correct anaemia found statistically significant improvement in locoregional tumor control following radiation therapy or radiochemotherapy.

Nonerythroid Function of EPO

Evidence indicates that the action of EPO is not limited to the erythroid system since EPO-Rs are expressed in numerous embryonic and adult tissues as well as in human cancers (38). EPO-Rs are present in nonerythroid blood lines including myeloid cells, lymphocytes, and megakaryocytes, as well as in multiple nonhaematopoietic cells, such as

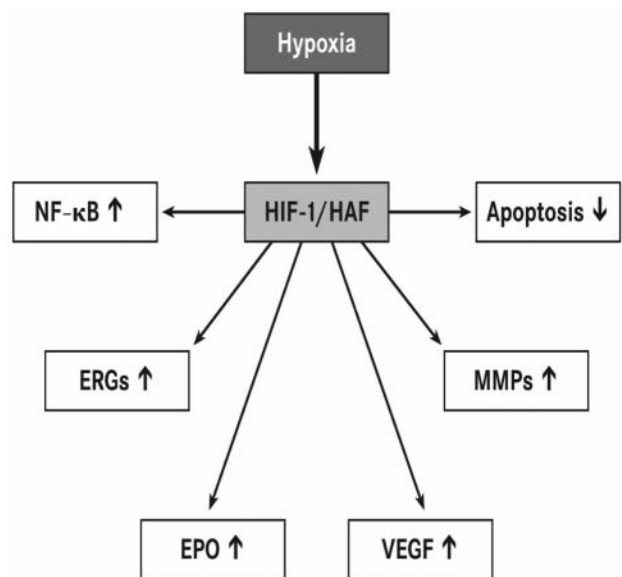


Figure 3. Hypoxia-induced expression of hypoxia-inducible factor-1 (HIF-1) in human cancers and activation of gene transcription. VEGF: vascular endothelial growth factor, MMP: matrix metalloproteinase, HAF: hypoxia-associated factor, NF-κB: transcription factor, ERGs: early-response genes.

endothelial cells; mesangial, myocardial, and smooth muscle fiber cells; neural cells, and renal cells (39) (Table III).

Moreover, many of these cell types exhibit active EPO signaling pathways and biological responses. For example, EPO stimulates survival and proliferation of endothelial cells *in vitro* and promotes new blood vessel formation *in vivo* (40). In a murine model, EPO stimulated angiogenesis in wound healing. In that model, macrophages in granulation tissue formed during wound healing expressed EPO-Rs, and EPO was shown to stimulate transforming growth factor (TGF-β1) production by activated macrophages. In mice expressing a constitutively active EPO-R, EPO signaling in endothelial cells was found to act in vascular repair (41). Most recently, Belenkov *et al.* (42) provided the first evidence that EPO can induce resistance in cancer cells *in vitro*. After addition of EPO to EPO-R expressing human glioblastoma, NSCLC and cervical cancer cell lines, cells became more resistant to cisplatin and ionizing radiation, and the effect was reversed by tyrphostin, an JAK2 inhibitor. The observation that the EPO application may cause resistance in cancer cells, if confirmed, will be of great clinical significance.

In brain neurons, a role for EPO-R signaling during ischaemia-associated neuronal angiogenesis has been proposed. Experiments have shown that the central nervous system responds to hypoxic insults by up-regulating the expression of EPO and the EPO-Rs by cells within the border zone surrounding the necrotic core of an injury over the 12-24 hours following the insult (43). Thus, evidence suggests that EPO acts not only as an endocrine hormone in the kidney/bone marrow system, but can act as a paracrine factor in the oviduct and the brain, possibly protecting against hypoxic injury, however, the underlying mechanisms of EPO as a neuroprotective agent are hypothesized to be multifactorial and not fully understood.

Clinical Studies

Anaemia is a common occurrence in patients with NSCLC and contributes to the clinical symptomatic and reduced quality of life seen in these patients. Many aspects of these symptoms are known to be associated with suboptimal low levels of haemoglobin. Several studies have found that inadequate oxygenation at the tumor site and/or low haemoglobin levels are associated with poor outcome after radio- or chemotherapy in patients with advanced cancers (44-46). On the other hand, adequate tumor oxygenation is known to be necessary for an optimal response to radiotherapy and chemotherapy (45), thus it is generally accepted that decreased haemoglobin levels (which may contribute to lowered oxygenation at the tumor site) have an effect on the efficacy of therapy. Prospective clinical trials in patients with cancer-related anaemia have

Table III. EPO-R expression and potential functions in normal nonerythroid cells [adopted from ref. (21)].

EPO-R expression	Function
Astrocytes	Decreased apoptotic cell death
Cardiomyocytes	Mitogenic
Endothelial cells	Mitogenic, endothelin-1 synthesis and release, angiogenic response (proliferation and migration)
Megakaryocytes	Maturation
Mesangial cells	Increased proliferation <i>in vitro</i>
Myeloid cells	Multilineage increase <i>in vitro</i> , immunomodulation
Neurons	Trophic effect, increased monoamine concentration, decreased apoptotic cell death
Renal cells	Mitogenesis
Prostate epithelial cells	Mitogenesis
Vascular smooth muscle cells	Contraction

determined that mild-to-moderate anaemia (haemoglobin level 8-12 g/dl) occurs in up to 75% of cancer patients undergoing treatment with chemotherapy and/or radiation therapy (47). The results of the European Cancer Anaemia Survey (ECAS) recently reported and confirmed the high prevalence of anaemia among patients with cancer (48). The ECAS enrolled 15,367 cancer patients at 748 cancer centers in 24 European countries; these patients were followed for up to six months to evaluate the incidence and prevalence of anaemia outside the clinical study setting. Overall, 72% of patients with haematological malignancies and 66% of patients with solid tumors were anaemic (haemoglobin level <12 g/dl) at some point during the 6-month survey. Of the 2,316 patients with multiple myeloma or lymphoma evaluable for analysis, 73% were anaemic at some point during the survey.

In lung cancer patients undergoing radiotherapy, anaemia is significantly associated with poorer local control of the disease and shortened survival times (49). Severe tumor-associated anaemia with low haemoglobin levels and/or considerable clinical symptoms has conventionally been treated with blood transfusions. Cancer patients with solid tumors receiving chemotherapy have been reported to require transfusions in 18% of cases. The highest rate of transfusion-dependent patients (34%) has been observed in patients suffering from lung cancer (50, 51). The main mechanisms involved in the development of chemotherapy-induced anaemia in NSCLC patients are cytotoxic bone marrow damage and renal impairment with subsequent decreased production of EPO. The first mechanism is induced by almost all cytotoxic drugs whilst the second has mainly been demonstrated following cisplatin treatment. NSCLC patients are generally treated with platinum-based

chemotherapy (Table I) and, therefore, both mechanisms are involved in the development of anaemia which subsequently occurs more frequently and more severely in NSCLC patients compared to other cancer patients (52). Platinum-containing chemotherapy protocols for the treatment of NSCLC patients induced grade ≥ 2 anaemia in 64 to 83% of all patients, with grade 3-4 anaemia occurring in 15-29% of patients treated (52).

New chemotherapy regimens are also associated with a high incidence of anaemia. Carboplatin-paclitaxel induced grade 3-4 anaemia in 34% of NSCLC patients with 30% of patients requiring blood transfusions (9). Similarly, 33% of NSCLC patients treated with cisplatin-gemcitabine received blood transfusions (52). Furthermore, EPO particularly benefits patients suffering from cisplatin-associated anaemia in which, in addition to other pathogenic mechanisms, nephrotoxicity may inhibit the adequate production of endogenous EPO (53).

Prevention of anaemia induced by intensive platinum-containing chemotherapy was studied in a randomized British trial (44): 36 patients with lung cancer were randomly assigned to three arms. They received EPO at a dosage of 150 U/kg three times per week or a dosage of 300 U/kg three times per week, both for the duration of chemotherapy, or they did not receive any EPO. Onset of anaemia was significantly delayed in both groups that received EPO. Furthermore, the requirements for red blood cell transfusions were significantly lower in EPO-treated patients than in untreated controls. In addition, there was a trend toward higher platelet counts and fewer platelet transfusions in patients undergoing EPO treatment. A similar study design was used in 21 cancer patients with cisplatin-associated anaemia. Again, EPO doses of 100 or 200 U/kg resulted in significant increases in haemoglobin levels within 4 weeks of treatment (53). Kunikane *et al.* (54) studied the clinical effect of EPO on anaemia induced by two courses of cisplatin-based chemotherapy in 72 patients with NSCLC. Patients were randomized into three groups, receiving 100, or 200 IU/kg EPO, or placebo. In 53 evaluable patients, haemoglobin levels were significantly elevated in the EPO groups suggesting that EPO can prevent anaemia in NSCLC patients who had cisplatin-based chemotherapy. A prospective phase II trial was carried out to evaluate the effectiveness of EPO in improving or maintaining performance status and haemoglobin levels in lung cancer patients treated with concurrent chemoradiation (55). A total of 51 patients with lung cancer (11 with SCLC, 40 with NSCLC), who underwent three different concurrent chemoradiation protocols, were enrolled. A significant increase was seen in the haemoglobin and performance status score ($p < 0.05$) in the final measurements suggesting that EPO may have a beneficial impact on these markers in patients undergoing

concurrent chemoradiation. Similar results have been reported from a prospective, open label, randomized trial (56). In this study 144 patients (mainly lung cancers) were enrolled. Patients in the treatment arm received 10000 U of EPO three times weekly during platinum-based chemotherapy, while patients in the control arm received no treatment. Transfusions were reduced by the administration of EPO (15.3 vs. 33.3%, $p = 0.019$), and fewer patients developed significant anaemia (16.6 vs. 45.8%, $p < 0.0001$). Most recently, Pradier *et al.* (46) published a phase II study where the effect of haemoglobin levels on the survival of NSCLC patients (stage III) was investigated: 56 patients were treated with concurrent low-dose cisplatin (6 mg/m², d 1-5, qw5-6) and thoracic radiotherapy (60 Gy plus 10 Gy boost). Patients with a pretreatment haemoglobin level ≥ 11.6 g/dl had a 2-year survival rate of 52% as compared to 15.5% for patients with haemoglobin levels < 11.6 g/dl ($p = 0.0075$), suggesting that haemoglobin levels prior to NSCLC therapy have a significant influence on prognosis and lower haemoglobin levels appeared to be associated with worse outcome. However, EPO administration is not only limited to platinum-based chemotherapy. In a recently published meta-analysis (57), patients from three controlled trials of patients with solid tumours receiving either platinum- or non-platinum-based chemotherapy, who had been randomised to EPO treatment or standard care were included ($n = 255$ and $n = 199$, respectively) to see if EPO was equally effective in both chemotherapy types. The primary endpoint was haemoglobin change. All patients responded rapidly to EPO treatment, showing a median haemoglobin increase of ≥ 1 g/dl from baseline at week 4 suggesting that the type of chemotherapy may not have an impact on the ability of EPO to rapidly increase haemoglobin in patients with solid tumours and chemotherapy-induced anaemia.

Taken together, these and other clinical studies have demonstrated that EPO is able to correct anaemia in nearly 60-80% of NSCLC patients receiving platinum-based chemotherapy and in approximately 40% of patients treated with regimens without platinum compounds, with a subsequent reduction in blood transfusion requirement (52). Despite evidence supporting the treatment of anaemia, many clinicians only intervene when haemoglobin levels fall below 8 g/dl (58). EPO is able to prevent the development of anaemia in NSCLC patients, suggesting that due to the high incidence of anaemia in this tumor type, EPO may represent an important tool in the supportive care of NSCLC patients. Although tumor-associated anaemia in NSCLC patients responds to EPO treatment in a dose-dependent fashion, treatment efficacy varies greatly among the different types of malignancies (Table IV). Response rates reported in the literature are not always directly comparable because response criteria have not been standardized. The highest response rates have been observed

Table IV. Responsiveness to EPO treatment.

Malignancy	Response rate
Head and neck cancer	86%
Multiple myeloma	77%
Esophageal cancer	75%
Lung cancer	67%
Colorectal cancer	50%
Ovarian cancer	50%
Chronic lymphocytic leukemia	50%
Breast cancer	45%
Non-Hodgkin's lymphoma	44%
Prostate cancer	40%
Hodgkin's disease	25%
Myelodysplastic syndrome	8%

in patients with lymphoproliferative disorders, with squamous cell carcinoma and with lung cancer (53).

In reliably predicting the response to EPO treatment, various potentially predictive factors have been studied, including pre-treatment EPO levels and changes in indicators of erythropoietic response measured 2 to 4 weeks after onset of treatment (58). However, although numerous variables (e.g. haemoglobin, ferritin) have been found to significantly correlate with response, none was associated strongly enough to serve as a reliable single prognostic factor (60). Of all factors studied, the change in haemoglobin levels after 2 weeks proved the most reliable. Thus, despite much research, highly reliable predictors of response to EPO have not been found, making targeting of EPO therapy to appropriate patients difficult. Furthermore, complications such as infections and functional iron deficiency, which can impair response to EPO, may further confound the problem (28).

To date, it is unclear whether low haemoglobin levels directly contribute to reduced tumor control and survival after radio- and chemotherapy, or whether it is merely a marker of advanced disease in patients with NSCLC for whom successful treatment outcomes are less likely, regardless of tumor oxygenation. However, a recent literature review suggests that anaemia is an independent prognostic factor in cancer patients, with the overall relative risk of death being 65% higher (95% confidence interval: 54-77%) in patients with anaemia than in patients without anaemia (61). Littlewood *et al.* (62) very recently reported a possible survival benefit in patients treated with EPO and chemotherapy, although these data are preliminary since the study was not designed to assess survival and the protocol did not control for variables that may influence survival. Similarly, preliminary data from trials of darbepoetin (NESP) in lung cancer patients receiving platinum-based chemotherapy suggest that median time to disease progression may be reduced with improvement of anaemia (13). Results from a study evaluating the activity of first-line

sequential high-dose chemotherapy for patients with testicular cancer showed a correlation between haemoglobin values after treatment completion and survival (63). In this study, patients with haemoglobin levels ≥ 10.5 g/dl after completion of four chemotherapy cycles had a 3-year overall survival rate of 87%, compared with 68% for patients with haemoglobin levels < 10.5 g/dl ($p < 0.03$). In contrast, Henke *et al.* (64) published a multicenter, double-blind, randomized, placebo-controlled trial with 351 head and neck cancer patients receiving radiotherapy (60-70 Gy). All patients were assigned to placebo (n=171) or EPO (300 IU/kg, n=180). Although in 82% of patients given EPO an increase of haemoglobin levels (> 14 g/dl) was achieved, loco-regional progression-free survival was poorer with EPO than with placebo, suggesting that EPO corrects anaemia but does not improve cancer control. However, it should be noted that this trial was attempting to treat patients prophylactically to keep haemoglobin levels higher than is usually recommended for patients with metastatic disease.

A recently published meta-analysis (27 randomized clinical trials, 3,287 patients) found a trend for improved survival in patients treated with EPO (65). In contrast to recent reports suggesting that EPO might negatively influence survival, these data suggest that EPO may have improved survival in the studied patients. Although at least 24 additional randomized clinical trials, ongoing or recently completed, were not part of this meta-analysis, the bulk of available information does not suggest a detriment to survival in cancer patients but rather a slight improvement in survival time.

EPO and NSCLC – Cost Analysis

Red blood cell transfusions have historically been reviewed as less expensive than EPO (66). A recent pharmaco-economic analysis comparing EPO administration with transfusions of blood in patients receiving chemotherapy for advanced cancer over a six-month period showed a 64% response rate to EPO at a cost of US\$ 12,971 compared to a 100% response rate to transfusion at a cost of US\$ of 4,481 (67). It is difficult, however, to gain a current, accurate measurement of the cost of acquiring, handling, processing, storing and administering blood, the costs associated with the complications of transfusions, and the indirect economic costs to patients due to travelling to a transfusion center and/or absence from work. One analysis placed the cost of collecting, testing, and administering blood, and treating complications at US\$ 324 per transfusion (68). A more recent retrospective study of 517 patients with haematological or solid tumors estimated the cost of a two-unit transfusion to be US\$ 938, but did not take into consideration the cost of treating complications (69). As the combination of fewer donations and increased demand decreases the supply of available blood and the number of

sophisticated screening tests rise, transfusion costs are likely to increase (70). It has been suggested that EPO administration may reduce the number of days NSCLC patients with anaemia spend in hospital. Hospitalization represents a significant burden on health care resources, as well as an inconvenience to the patient. Therefore, consideration of treatments that lighten this load is important.

Pharmacoeconomic analyses are clearly needed that incorporate: (a) the cost of transfusions (handling and administering blood, and managing complications); (b) the economic consequences of lost productivity associated with administering transfusions; (c) the cost of administering EPO once the optimal dose and schedule is defined; and (d) the cost of administering EPO once the physicians are able to target therapy to those NSCLC patients most likely to respond and/or terminate therapy once a response is ruled out. Furthermore, the economic costs of lost productivity by patients who are unable to work due to symptomatic anaemia must be considered and the psychological costs of patient preferences to avoid transfusions should also be taken into account.

EPO and NSCLC – Recommendations

Anaemia is widely believed to be under-recognized and under-treated (71). Most physicians do not administer transfusions until severe anaemia develops (haemoglobin below 8 to 9 g/dl) (72-74) and a recent survey of 3,472 patients treated by 20 community oncologists found that 52 to 70% of anaemic patients (haematocrit <30%) undergoing chemotherapy did not receive treatment with EPO (75). There are several possible explanations why anaemia is undertreated: lack of awareness of the incidence and impact of anaemia; inadequacies in the current treatment options; and the existence of several key uncertainties surrounding the management of anaemic cancer patients.

Monitoring, identifying and effectively treating anaemic NSCLC patients is not an easy task. Physicians must consider many issues on a regular basis to ensure that their patients do not develop and subsequently suffer from the effects of anaemia. An issue that is currently unclear is exactly how to determine an appropriate threshold for therapeutic intervention. That is, if a patient's haemoglobin level is decreasing, which criteria should be used to determine the point at which the patient requires therapy? Clearly, physical symptoms should be considered as well as haemoglobin values. The study by Cleland *et al.* (75) demonstrated the greatest benefits for cancer patients when haemoglobin levels increase from 11 to 12 g/dl. This suggests that a haemoglobin level at or below 11 g/dl may be an appropriate threshold in those NSCLC patients whose symptoms have not already necessitated intervention. However, as some patients experience the effects of

anaemia before their haemoglobin falls below 11 g/dl, it seems unlikely that a standard haemoglobin value as a cut-off point will identify all NSCLC patients who could benefit from EPO therapy (76).

It has been recommended that an initial dose of EPO of 150 U/kg should be given three times weekly subcutaneously, which is increased to 300 U/kg if the haemoglobin levels do not increase by ≥ 1 g/dl after 4 weeks of therapy. More and more, EPO is typically administered once weekly at a dose of 40000 U/dose. Although the two schedules have not been compared in a randomized trial, weekly dosing has the benefit of reduced frequency of administration and greater patient convenience. A NSCLC patient who does not respond to the doubled dose is unlikely to respond to higher doses (77) and should therefore have therapy terminated at this point. However, whether this treatment recommendation is being followed in routine clinical practice or whether it constitutes the most efficient and effective use of EPO is unclear. Thus, it is prudent to consider both symptoms and haemoglobin values in NSCLC patients before making treatment decisions. However, the relative value placed on each parameter remains to be defined. Recently, a new dose-intense EPO regimen in anaemic cancer patients has been proposed (78). Twenty-nine patients with non myeloid malignancies received a single induction *s.c.* dose of EPO 40000 U day 1 and two doses of 10000 U *s.c.* on days 3, 5, 8, 10, 12, 15, 17 and 19. Iron supplementation (125 mg *i.v.*) was also performed. The results of this study suggest that an induction dose of 40000 U of EPO, followed by 8 maintenance doses of 20000 U each, may improve the standard response in terms of both time to response and haemoglobin increase. Moreover, the haemoglobin levels seemed to increase after EPO therapy discontinuation (d22-29). In addition, Mystakidou *et al.* (79) have provided evidence that patients may benefit from iron supplementation during EPO administration. A total of 100 patients with cancer-related anaemia, not subjected to chemotherapy and/or radiotherapy, were randomised to receive, for a maximum of 24 weeks, either oral iron (200 mg), or EPO 40000 U *s.c.* once weekly plus oral iron once daily. Patients in the EPO group had, from baseline to study end, a mean increase in haemoglobin levels of 2.4 g/dl, whereas in the control group the mean haemoglobin level decreased by 0.1 g/dl ($p < 0.001$), suggesting that this regimen may offer optimal therapy in this population taking into consideration physician's convenience and patient's compliance.

Whatever the outcome, NSCLC patients should not have to wait until their anaemia becomes debilitating to receive treatment. As studies show that many, but not all, NSCLC patients experience anaemia (80), it would be of great benefit to be able to predict whether a patient is at high risk of developing anaemia. Such knowledge would assist physicians in monitoring patients more closely and/or

administering EPO before anaemia becomes symptomatic. The results of a study by Ray-Coquard *et al.* (61) may assist in this goal; however, this requires validation in a large, multicenter study.

Finally, there are no universally accepted guidelines addressing the most effective methods of monitoring NSCLC patients for anaemia, and, once identified, managing the condition. The development of such guidelines may assist in improving current treatment protocols and result in more widespread and effective management of anaemic patients with NSCLC.

Future Directions

The myelosuppressive toxicities of chemotherapy are one of the principle reasons for the overall failure of some agents to have a meaningful impact on responses and survival in cancer, and anaemia is a common side-effect of almost all cytostatic drugs used clinically. Although EPO administration is known to correct CRAs, one of the downsides to EPO use is that across all studies the typical response rate is approximately 64% for all patients treated (25). To date, it remains unclear which mechanisms may contribute to this limitation of EPO therapy, although cytokine-mediated suppression of the haematopoietic progenitors has been hypothesized (49). In addition, a typical patient receiving EPO will have a delayed response that can take as long as 4 to 8 weeks (81). Currently, newer options focusing on increasing the response rate as well as the time to response are being evaluated. Once-weekly EPO dosing has been adopted by oncologists worldwide and has been incorporated into treatment guidelines (82). Data from some pilot studies suggest that higher weekly starting doses of EPO may allow patients to more quickly achieve a 2 g/dl increase in haemoglobin level or a >12 g/dl target haemoglobin level compared with EPO at a dose of 40000 U/week (83). In addition, these results establish the feasibility of less frequent maintenance dosing in anaemic cancer patients undergoing chemotherapy. Alternative dosing regimens may ultimately provide flexibility for prescribers in selecting a dose and schedule of administration based on individual patient needs and convenience, without comprising efficacy. A variety of EPO doses and dosage regimens are being currently investigated in larger clinical trials. Another approach uses a novel erythropoiesis stimulating protein (NESP). NESP is a genetically modified EPO protein with a prolonged half-life compared with native EPO (84, 85). In a recently published study (13), NESP therapy seems to be associated with a statistically significant improvement of haemoglobin levels and a reduction in the need for RBC transfusion. Furthermore, the data suggest that NESP patients with lung cancer had a longer progression-free survival than did those on placebo. It is anticipated that additional studies evaluating

the value of NESP compared with standard EPO will be conducted to determine whether less frequent dosing with NESP results in more rapid recovery or higher response rates. The possibility that the normalization of haemoglobin levels with EPO or its derivatives may play a role in improving cancer chemotherapy survival is currently being studied in a number of randomized trials and, if confirmed, will be of potential clinical significance. Substantial clinical and economic benefits could be provided by the ability to deliver EPO by gene therapy rather than by repeated injections. In an animal model, transplantation of autologous vascular smooth muscle cells, transduced with a retroviral encoding EPO-cDNA, induced vector-derived EPO secretion while endogenous EPO-mRNA was largely down-regulated in the kidney. Compared with untreated controls, reticulocyte counts increased and clinically significant increases in both haematocrit and haemoglobin levels were observed (86). If a reliable technique could be developed to control expression of such therapeutic EPO genes, EPO gene therapy might become a promising treatment alternative in EPO-responsive patients with tumor-associated anaemia in NSCLC patients.

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