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Review

Preclinical studies of erythropoietin receptor expression in tumour cells: Impact on clinical use of erythropoietic proteins to correct cancer-related anaemia

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ABSTRACT

In vitro and animal model studies have shown erythropoietin receptor (Epo-R) mRNA and/or protein may be present in a range of human tumours and cancer cell lines, and erythropoiesis-stimulating agents (ESAs) have been reported to have tumour cell growth-modulating effects. Following a review of the literature, we conclude that considerations must be made when interpreting data from the preclinical studies. First, supraphysiological doses of ESAs were usually used. Second, there are no well validated, commercially available antibodies for identifying the presence and functionality of Epo-R at the protein level, either intracellularly or on the cell surface. Data from previous studies that used antibodies only for Epo-R detection must therefore be interpreted with caution. Together with diverging results in the literature, these methodological limitations indicate that findings from preclinical studies must not be over-translated in terms of their clinical relevance to patients with cancer.

1. Introduction

Erythropoietin (Epo) is a 30.4 kDa glycoprotein hormone that regulates erythropoiesis by stimulating growth, preventing apoptosis, and inducing differentiation of red blood cell precursors.[1] Impaired production of Epo or a blunted response to its production decreases the number of circulating red blood cells and consequently anaemia develops. One of the most debilitating symptoms of anaemia is fatigue, which can adversely affect a patient's quality of life.[2] Also, anaemia

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is increasingly recognised as a negative prognostic and predictive factor for patients with a wide variety of tumours undergoing chemotherapy, radiation therapy, or a combination of these treatments.[2]

Erythropoiesis-stimulating agents (ESAs) are used in the treatment of patients with anaemia due to cancer or cancer therapy, as well as anaemia due to renal failure and other chronic conditions.[3] Three different ESAs are available commercially: epoetin alfa, epoetin beta, and darbepoetin alfa. It has been shown in several randomised, placebo-controlled studies of patients with chemotherapy-related anaemia that ESA therapy reduces transfusion requirements, increases haemoglobin (Hb) concentration, and improves quality of life.[4–7]

The Epo receptor (Epo-R) was first observed in erythroid cells.[8] These studies showed that the number of receptors located on the cell surface of erythroid cells is low, ranging from a few hundred to a few thousand receptors per cell. In recent years, the expression of Epo and its receptor has been noted in several non-haematopoietic tissues and cells, including the liver, uterus, central nervous system, vascular endothelial cells, and also in malignant tumours.[9,10] Furthermore, Epo has been shown to exhibit diverse biological effects, such as neuro- and cardio-protection against anoxic injury[11,12] and stimulation of physiological angiogenesis in the uterus, in proliferative diabetic retinopathy, and during wound healing.[13–15]

Observations of Epo-R expression in cancer cells, coupled with identification of non-haematopoietic functions of Epo, have stimulated much preclinical research into the potential growth-modulating and hypoxia-related effects of Epo on cancer cells. Although these studies have undoubted scientific interest, it is important to consider their relevance to the clinically approved use of ESAs in the treatment of cancer-related anaemia. Parallels can be drawn with the clinical experience of recombinant colony-stimulating factors (CSFs) used to treat neutropenia associated with cancer therapy. Receptors for CSFs have been identified on a variety of cancer cell types[16–18], but there is no evidence from clinical trials or clinical experience with recombinant CSFs to suggest adverse effects on tumour progression.[19]

A recent meta-analysis of 42 trials with 8167 patients suggested that treatment of anaemia with an ESA may have no impact on overall survival in patients with cancer.[20] In contrast, two randomised studies demonstrated poorer outcomes with ESA therapy compared with placebo in patients with head and neck cancer receiving radiotherapy[21] and in patients with breast cancer receiving chemotherapy.[22]

This article critically reviews current evidence of Epo and Epo-R expression in cancer cells in the context of tumour progression and effects on hypoxia. The preclinical data are examined and related to clinical trial results in order to assess the relationship between ESA therapy and tumour cell growth and survival. The need to consider the implications of methodological limitations in many preclinical studies is highlighted from the outset.

2. Epo, Epo-R, and tumour progression: preclinical studies

There are several mechanisms postulated by which Epo could positively or negatively influence tumour progression, including direct growth-regulatory effects, modulation of tumour hypoxia, effects on the efficacy of radiation and chemotherapy, and impact on angiogenesis. Data available from published preclinical studies of ESAs demonstrate potential positive, negative and, in some cases, no effects via each of these pathways. Before describing these data, however, there are two important considerations to be made, which are likely to affect interpretation of the preclinical study results.

2.1. *Detection and measurement of Epo-R*

There are no well-validated techniques for identifying Epo-R on the cell surface or intracellularly and those used most frequently have limitations.[23] Reverse transcriptase-polymerase chain reaction (RT-PCR) requires isolation of malignant cells from surrounding tissue, with the potential for contamination by non-tumour cells. Also, RT-PCR is usually regarded as a very sensitive, qualitative (rather than quantitative) measure, and only detects Epo-R transcripts, not functional mRNA or receptor protein. For example, using RT-PCR, Westphal and colleagues investigated Epo-R and granulocyte-CSF (G-CSF) expression in human tumour cells.[24] Genomic Epo-R expression was detected in various tumour cell lines of non-haematopoietic origin but additional analyses of epoetin-induced cellular proliferation revealed no effect on the growth of tumour cell lines, highlighting the importance of investigating the functionality as well as the presence of Epo-R at the protein level. Similar findings have recently been described in lymphoid malignancies.[25]

Antibody-based techniques have major limitations for detecting functional protein receptors. Immunohistochemistry has been reported to detect Epo-R in the cytoplasm as well as on the cell surface but has limitations related to the reagents used to detect functionality. Importantly, the commercially available anti-Epo-R antibodies have not been characterised sufficiently in terms of specificity and selectivity to allow clear identification of Epo-R expression over background staining. This is most clearly demonstrated by a recent study in which all but one commercially available anti-Epo-R antibody was shown to lack specific Epo-R staining using Western blot analysis.[26] The only antibody that detected the Epo-R (M-20, Santa Cruz Biotechnology) did so by immunoblotting but not by immunohistochemistry. The same research group also investigated if Epo-R protein expression correlated with surface expression of the receptor in breast adenocarcinoma and neuroblastoma cell lines.[27] Although high levels of Epo-R protein were detected by Western blot, there was no detectable ^{125}I Epo binding, questioning the presence of surface Epo-R. Similar findings were reported in a preliminary study of tumour cells from patients with lymphoproliferative tumours.[25]

These results indicate that all previous publications on Epo-R protein expression must be viewed with great caution.

2.2. *ESA doses*

An important consideration when evaluating preclinical evidence is the *in vitro* dose used. The normal serum concentration range for endogenous Epo in non-anaemic, healthy individuals is in the order of 0.005–0.025 U/ml. In many of

the preclinical studies evaluating the potential effects of ESAs on cancer cells, the doses tested were several orders of magnitude higher than both the physiological concentration of endogenous Epo and the levels obtained in patients following administration of exogenous ESA (Tables 1 and 2). Typically, modulatory effects were seen only at the highest ESA concentrations used in the experiments.

2.3. *Direct effects on tumour cell growth, survival, and migration*

Given the methodological limitations discussed above, Epo-R mRNA and/or protein expression have been reported in a range of cancer cell lines, including human hepatocarcinoma, renal carcinoma, melanoma, breast carcinoma, malignant tumours of female reproductive organs, non-small cell lung carcinomas, and vestibular schwannoma (Table 1).[28,32–41] Other studies have reported Epo binding or Epo-R protein in biopsies of human lung, head and neck, breast, endometrial, and uterine cervix carcinomas.[28–31,42,43,52–55] Although Epo-R may be expressed by a number of tumour cell lines, *in vitro* and animal model studies do not provide conclusive evidence that ESAs stimulate tumour cell proliferation. Initial *in vitro* studies failed to show growth-modulating effects of ESAs in a number of tumour cell lines, even in cells reported positive for the Epo-R.[24,56–60] One study investigated tyrosine kinase activity in Epo-R-positive cell lines, an Epo-R-negative cell line, and the Epo-dependent haematopoietic cell line, UT-7.[24] The UT-7 cell line showed an increase in tyrosine kinase activity after incubation with epoetin for up to 30 min. None of the other cell lines showed induction of tyrosine kinase activity, despite the high concentration of Epo used.

Other *in vitro* and animal model studies suggest that ESAs may stimulate tumour cell proliferation.[28,38,41] However, supraphysiological doses were used in two of these studies.[28,38] The third study used erythropoietin mimetic protein (EMP) 9, an antagonist to the Epo-R, and EMP1, an Epo mimetic.[41] Other studies have demonstrated tumour regression following local injection of Epo antibodies or soluble forms of the Epo-R into tumour tissue.[39,40] In contrast, epoetin treatment was shown to induce tumour regression in murine myeloma models.[61] Also, two other recent animal model studies found no differences in tumour growth between epoetin-treated and placebo-treated groups of rodents transplanted with rodent or human tumour cell lines that expressed Epo-R.[51,62] These latter findings suggested that, although Epo-R may be expressed, it was not involved in tumour growth promotion.

2.4. *Role of tumour hypoxia*

Solid tumours are characterised by regions of hypoxia due to the long oxygen diffusion distance between the incomplete vasculature and the mass of oxygen-consuming cells.[63] Hypoxia induces the transcription of several genes encoding proteins that favour tumour cell survival and tumour growth.[63,64] Among these proteins are the angiogenic vascular endothelial growth factor (VEGF) and several glycolytic enzymes. It is also well established that anaemia-induced tumour hypoxia may result in reduced sensitivity to radiotherapy; correction of anaemia with an ESA may therefore theoretically improve tumour oxygenation.

In vitro studies have demonstrated a correlation between hypoxia and expression of Epo and the Epo-R. A study of 21 patients with squamous cell carcinoma of the head and neck investigated expression of Epo and Epo-R, and the association between Epo expression and tumour hypoxia.[53] Using immunohistochemistry techniques, co-expression of Epo and Epo-R was reported in 90% of biopsies, and a significant positive correlation was noted between levels of microregional Epo expression and binding of pimonidazole, a marker of hypoxia. A similar study conducted by the same research group in 38 patients with breast cancer also found a significant positive correlation between levels of microregional Epo expression and pimonidazole binding.[54] Additionally, correlation was found between levels of Epo-R expression and pimonidazole binding. Whether these findings indicate that Epo may function as a para- or autocrine growth factor in head and neck or breast cancer is not known.

Using immunohistochemistry, another study evaluated Epo-R expression in human head and neck carcinomas and its correlation with tumour hypoxia and treatment outcome.[65] While Epo-R expression was reported in the majority of tumours, no colocalisation was found between Epo-R and pimonidazole binding. Moreover, the level of Epo-R expression was not correlated with treatment outcome. Similarly, a study of head and neck squamous cell carcinoma found the expression of Epo, but not Epo-R, correlated with the expression of hypoxic-dependent proteins.[66] There was no survival difference between patients with high and low Epo or Epo-R expression.

Preclinical studies have reported possible effects with ESA treatment on tumour hypoxia. In a study of human breast cancer cell lines, Epo and Epo-R levels were reported to be up-regulated by hypoxia, and epoetin was shown to stimulate DNA synthesis and cell proliferation (Table 2).[28] Another study demonstrated improved tumour oxygenation following administration of epoetin to rats transplanted with a rodent mammary carcinoma.[67] In rats randomised to epoetin after tumour implantation, epoetin before tumour implantation or placebo, this improvement was independent of effects on haematocrit levels.[67] Mean haematocrit was equal between arms at therapy initiation but was significantly higher for both arms receiving epoetin at day 22 ($P = 0.052$). However, in the group that received epoetin after tumour implantation, tumours were significantly less hypoxic when compared with either placebo or those receiving epoetin before tumour implantation.

2.5. *Effects on tumour sensitivity to radiation and chemotherapy*

A related area of investigation is the effect of ESAs on the efficacy of radiation or chemotherapy, with conflicting results reported in *in vitro* studies (Table 2). In one study, human cervical cancer cells (HeLa) were pre-treated with epoetin (25, 50, or 200 U/ml) for 1 h and then challenged with cisplatin for 20 h.[43] Using a non-radioactive cell proliferation (MTT) assay, a dose-dependent effect was observed in the fraction of surviving cells. This study also found a significant correlation between expression of the Epo-R and the anti-apoptotic protein

Table 1 – Summary of preclinical studies reporting Epo receptor (Epo-R) expression and possible direct effects on human tumour cell growth and survival with ESA treatment

Study	Disease type	Methods for detection of Epo-R	ESA dose used	Main findings
Acs et al.[28]	Breast cancer cell lines and breast cancer biopsies	Immunohistochemistry RT-PCR Western blotting	250 U/ml	Epo and Epo-R expressed by breast cancer cells. Cell lines proliferated in response to ESA
Acs et al.[29]	Breast cancer biopsies	Immunohistochemistry	na	Epo and Epo-R expressed by breast cancers; Epo-R immunostaining increased in carcinomas
Acs et al.[30]	Endometrial cancer samples	Immunohistochemistry	na	Epo and Epo-R expressed by endometrial cancers
Amin et al.[31]	NSCLC samples	Immunohistochemistry	na	Epo and Epo-R expressed by NSCLC; co-expression in majority of tumours
Arcasoy et al.[32]	Prostate cancer cell lines and biopsies	Immunohistochemistry RT-PCR Western blotting	na	Epo and Epo-R co-expressed in prostate cancer cell lines
Dagnon et al.[33]	NSCLC samples	Immunohistochemistry RT-PCR	na	Epo and Epo-R expressed by NSCLC
Dillard et al.[34]	Intracranial cancer samples (vestibular schwannoma)	Immunohistochemistry	na	
McBroom et al.[35]	Epithelial ovarian carcinoma cells	Immunohistochemistry Western blotting	na	Epo-R expressed by ovarian carcinoma cells
Ohigashi et al.[36]	Hepatocellular carcinoma cells	Radiolabelled ESA	5–80 mU/ml	Epo and Epo-R expressed by hepatocellular carcinoma cells
Selzer et al.[37]	Melanoma cell lines	RT-PCR Western blotting	na	Epo-R is expressed in transformed human melanocytes
Westenfelder & Baranowski[38]	Biopsies of human RCC, human and mouse renal cancer cell lines	RT-PCR Western blotting	0.5–100 U/ml	ESA stimulates proliferation of human renal carcinoma cells
Yasuda et al.[39]	Xenografts of ovarian and uterine cancers	Immunohistochemistry RT-PCR	na	Inhibition of Epo signalling destroyed xenografts in nude mice
Yasuda et al.[40]	Biopsies of female reproductive tumours	Immunohistochemistry RT-PCR	na	Inhibition of Epo signalling reduced growth and/or survival of transformed cells and capillary endothelial cells
Yasuda et al.[41]	Xenografts of human stomach choriocarcinoma and melanoma cell lines	Immunohistochemistry RT-PCR	na	Survival of tumour cells inhibited following injection of Epo-R antagonist; survival promoted with Epo mimetic peptide

Epo, erythropoietin; Epo-R, erythropoietin receptor; ESA, erythropoiesis-stimulating agent; na, not applicable; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; RT-PCR, reverse transcriptase-polymerase chain reaction.

Table 2 – Summary of preclinical studies reporting effects with ESA treatment on human tumour hypoxia, sensitivity to radiation and chemotherapy, and angiogenesis

Study	Disease type	Methods for detection of Epo-R	ESA dose used	Main findings
Effects on tumour hypoxia				
Acs et al.[28]	Breast cancer cell lines and breast cancer biopsies	Immunohistochemistry RT-PCR Western blotting	250 U/ml	Epo and Epo-R levels up-regulated by hypoxia
Acs et al.[42]	Breast cancer cell line	RT-PCR Western blotting	200 U/ml	Autocrine Epo signalling inhibits hypoxia-induced apoptosis
Effects on tumour sensitivity to radiation and chemotherapy				
Acs et al.[43]	Cervical cancer cell lines and biopsies	Immunohistochemistry RT-PCR Western blotting	0–200 U/ml	ESA inhibited cytotoxic effect of cisplatin
Belenkov et al.[44]	Glioma and cervical cancer cell lines	Immunohistochemistry Western blotting	30 U/ml	ESA induced resistance to ionising radiation and cisplatin
Carvalho et al.[45]	Renal cancer and myelomonocytic leukaemia cell lines	Immunofluorescence, cytofluorometric analyses	5 and 10 U/ml	ESA enhanced apoptotic response to daunorubicin and vinblastine
Gewirtz et al.[46]	Breast cancer and leukaemic cells	RT-PCR	10 U/ml	No significant change in effectiveness of taxol, tamoxifen or adriamycin in breast tumour cells
Kumar et al.[47]	Melanoma cell lines	Immunohistochemistry Western blotting	10 and 100 U/ml	ESA treatment increased resistance to hypoxia and CT drugs
Liu et al.[48]	Variety of malignant cell lines	Flow cytometry	10 U/ml	No significant change in sensitivity to cisplatin despite presence of Epo-R
McBroom et al.[35]	Epithelial ovarian carcinoma cells	Immunohistochemistry Western blotting	0, 25, 50, 200 U/ml	ESA treatment increased resistance to CT drug
Pajonk et al.[49]	Transfected HeLa cervical cancer cells	RT-PCR Western blotting Flow cytometry	25 U/ml	ESA treatment increased clonogenicity of Epo-R-expressing cells but did not alter radiation sensitivity
Effects on angiogenesis				
Batra et al.[50]	Cell lines and primary tumour cells from paediatric cancers	Immunohistochemistry RT-PCR Flow cytometry	10 or 30 U/ml	ESA increased expression of anti-apoptotic genes, and production/secretion of angiogenic growth factors
Hardee et al.[51]	Two rodent cancer cell lines, human colon carcinoma, human H& N tumour	Immunohistochemistry	100 U/ml (2000 U/kg three times weekly)	No difference in vascular length density between placebo- and ESA-treated groups

CT, chemotherapy; Epo, erythropoietin; Epo-R, erythropoietin receptor; ESA, erythropoiesis-stimulating agent; H&N, head and neck; RT-PCR, reverse transcriptase-polymerase chain reaction.

bcl-2 ($R = 0.4632$, $P < 0.0001$). However, in a study of seven cancer cell lines, a lower dose of epoetin (10 U/ml) had no effect on bcl-2 expression.[48] In addition, the preculture of cells with epoetin did not cause any significant change in sensitivity to subsequent exposure to cisplatin, despite the reported presence of Epo-R in all but one of the cell lines.

An adverse effect of ESAs on the sensitivity of various cancer cell lines to cisplatin was reported in two recent studies.[34,44] In contrast, other recent studies have demonstrated either no effect[46] or a sensitising, pro-apoptotic effect of ESA treatment when used in combination with chemotherapy agents.[45,68] In the study by Carvalho and colleagues, human renal carcinoma and myelomonocytic leukaemia cell lines cultured in the presence of epoetin (5 U/ml) exhibited an elevated apoptotic response to daunorubicin and vinblastine.[45] The chemosensitisation effect of epoetin was suggested to result from inhibition of the NK-κB rescue pathway. In another study, activation of the same pathway with epoetin had no effect on intrinsic radiation sensitivity of cervical (HeLa) cancer cells.[49] However, in comparison with tumour cells not expressing Epo-R, there was an increase of more than 50% in the number of clonogenic tumour cells if HeLa cells expressed Epo-R and were stimulated with epoetin.

Studies in animal models have demonstrated enhanced sensitivity to chemotherapy following correction of chemotherapy-induced anaemia with an ESA.[69,70] In a study of rats, one group had anaemia induced with carboplatin, and in the second group, anaemia was prevented with epoetin treatment.[70] Neither carboplatin nor epoetin treatment influenced tumour growth rate. However, after treatment with cyclophosphamide, the growth delay was significantly shorter in the anaemic group compared with non-anaemic controls (8.6 *versus* 13.3 days, $P < 0.05$).

2.6. *Effects on tumour angiogenesis*

The identification of Epo-R expression in endothelial cells[71] warranted investigation of an association between Epo administration and angiogenesis (Table 2). A study of tumour cells from common paediatric cancers demonstrated increased production and release of angiogenic growth factors, VEGF and/or placenta growth factor (PLGF), following treatment with high doses of epoetin (10 or 30 U/ml).[50] Another study, which examined Epo signalling in xenografts of human stomach choriocarcinoma and melanoma cell lines, reported inhibition of angiogenesis and decreased tumour cell survival when Epo signalling was blocked with an Epo-R antagonist.[41] In contrast, an Epo mimetic peptide promoted angiogenesis and tumour cell survival. However, there was no effect on angiogenesis in a study of epoetin-treated rats transplanted with rodent or human tumour cells.[51] In the mammary window chamber system, there was no difference in vascular length density between placebo- and epoetin-treated groups at any timepoint.

3. ESAs and tumour progression: the clinical evidence

Observations that low Hb levels are associated with poor prognosis in patients with cancer raised the question of whether correction of anaemia may result in improved outcomes. Clinical improvement has been demonstrated in several randomised, placebo-controlled studies of patients with solid tumours and lymphoproliferative malignancies; treatment with ESAs raised Hb levels, reduced transfusion requirements, and improved quality of life.[4–7]

At present, there is no clear evidence to suggest that ESA treatment improves or has a detrimental effect on overall survival (Table 3). A meta-analysis of studies published between January 1985 and December 2001 that included 19 trials of 2805 adult patients with cancer showed that outcomes may be improved in the epoetin-treated group.[81] However, a later follow-up of the Cochrane analysis, involving 42 trials of 8167 patients, revealed no differences between epoetin/darbepoetin-treated and untreated groups.[20] Some clinical trials have indicated a trend towards improved survival in patients treated with epoetin or darbepoetin.[6,72,74] Also, a significant improvement in disease-free survival was reported in a study of patients with pelvic malignancies receiving epoetin and radiotherapy.[73] Another study, in patients with high-risk cervical cancer receiving sequential adjuvant chemo-radiotherapy, demonstrated significantly better relapse-free survival in patients receiving epoetin compared with a control group receiving transfusions as required.[78]

As well as the updated Cochrane analysis,[20] other studies reported no significant difference in survival between patients with cancer receiving and those not receiving ESA treatment (Table 3).[75,76,85] No significant impact on survival compared with placebo was observed after long-term follow-up of a randomised study of epoetin beta in anaemic patients with lymphoproliferative malignancies.[84] A meta-analysis of randomised controlled trials of epoetin beta in anaemic patients with cancer identified a trend towards a reduced risk of disease progression, and no suggestion of decreased survival.[77] Also, meta-analyses conducted on trials of epoetin alfa and darbepoetin alfa indicate similar overall survival in cancer patients receiving an ESA compared with placebo.[79,82]

Importantly, none of the randomised clinical trials has reported a difference between ESA and placebo with regard to the proportion of patients with progressive disease while on therapy, suggesting ESA treatment has no tumour-promoting effect *in vivo*. However, two recent phase III studies in which survival was a primary endpoint have raised concerns that ESAs may have a significant negative impact on survival (Table 3).[21,22]

In the study of epoetin beta in patients with head and neck cancer undergoing radiotherapy, survival differences were limited to a subgroup of patients with hypopharyngeal cancer.[21] Also, after 9 weeks of epoetin therapy, mean Hb levels were 15.4 ± 1.7 g/l for epoetin-treated patients indicating that many patients were overcorrected. Theoretically, overcompensation may lead to increased viscosity and a reduced oxygen supply to the irradiated tumour areas.[86,87] In addition, there were differences at baseline between groups in several prognostic factors that may have favoured better outcome in the placebo group, including smoking status (66% were current smokers in the epoetin group *versus* 53% in the placebo group), tumour relapse before treatment (10% *versus* 8%), and tumour-node-metastasis (TNM) stage IV (75% *versus* 72%, based on AJCC staging system). These differences between

Table 3 – Summary of clinical trials reporting effects of ESA treatment on tumour progression or overall survival in patients with cancer

Study	Cancer type	Number of patients	Type of cancer treatment	Effect of ESA
Studies showing positive/neutral effect on disease progression or survival				
Littlewood et al.[6]	Solid and non-myeloid haematological	375	CT	Overall survival: HR 1.309 ($P = 0.052$) in favour of ESA *versus* placebo
Glaser et al.[72]	H&N	191 (144 with pre-treatment Hb <14.5 g/l)	CT, RT	Patients with pre-treatment Hb <14.5 g/l: significant improvement in 2-year locoregional control and 2-year survival and control ($P \leqslant 0.001$)
Antonadou et al.[73]	Pelvic	385	RT	4-year disease-free survival: 85.3% and 67.2% in ESA and control group, respectively ($P = 0.0008$)
Vansteenkiste et al.[74]	Lung	320	CT	Trend toward improved progression-free and overall survival *versus* placebo
Bamias et al.[75]	Various solid	144	Pt-CT	No significant difference in disease progression
Rosen et al.[76]	H&N	90	CT, RT	No significant difference in overall and progression-free survival *versus* control
Aapro et al.[a77]	Various	1409	Various	Trend toward reduced risk of progression *versus* control (RR = 0.79, 95% CI = 0.62–1.00). No effect on overall survival
Blohmer et al.[78]	Cervical	257	CT, RT	Relapse-free survival significantly better *versus* control (19 *versus* 31 events, $P = 0.034$) No difference in overall survival
Johnson & Johnson[a79]	Various	1976	Various	No difference in overall survival *versus* placebo (HR = 0.99, 95% CI = 0.76–1.28)
Machtay et al.[80]	H&N	135	RT, CT	No difference in loco-regional control or overall survival between groups
Bohlius et al.[a81]	Various	2805	Various	Trend toward improved overall survival *versus* control (HR = 0.81, 95% CI = 0.67–0.99)
Bohlius et al.[a20]	Various	8167	Various	No difference in overall survival *versus* control (HR = 1.08, 95% CI = 0.99–1.18)
Hedenus et al.[a82]	Lung, lymphoproliferative	1129	CT	No difference in overall survival *versus* placebo (HR = 0.95, 95% CI 0.78–1.16)
Michael et al.[83]	Breast	1143	CT	No difference in disease-free or overall survival *versus* control
Österborg et al.[84]	CLL, NHL, MM	349	Various	No significant difference in overall survival *versus* placebo
Witzig et al.[85]	Various	330	CT	No significant difference in overall survival *versus* placebo
Studies showing a negative effect on disease progression or survival				
Henke et al.[21]	H& N	351	RT	Increased locoregional progression (RR 1.69, $P = 0.007$) and decreased survival (RR 1.39, $P = 0.02$) *versus* placebo
Leyland-Jones et al[22]	Breast	939	CT	12-month overall survival decreased *versus* placebo (70% *versus* 76%, $P = 0.01$)

CI, confidence interval; CLL, chronic lymphocytic leukaemia; CT, chemotherapy; ESA, erythropoiesis-stimulating agent; Hb, haemoglobin; H&N, head and neck; HR, hazard ratio; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; Pt-CT, platinum-based chemotherapy; RR, relative risk; RT, radiotherapy.
a Meta-analysis.

groups were even more apparent in patients with hypopharyngeal cancer (current smoker: 55% in the epoetin group *versus* 40% in the placebo group; relapse before treatment: 15% *versus* 7%; and stage IV disease: 85% *versus* 70%, respectively). However, the results of this trial must be considered seriously until data from additional trials on ESA treatment in head and neck cancer patients are available.

Possible limitations of the study of epoetin alfa in patients with metastatic breast cancer receiving chemotherapy[22] have also been identified, with the investigators highlighting a lack of standard assessment and documentation of important prognostic factors for survival.[88] Also, optimal tumour response and time to disease progression were similar between groups and the difference in survival was attributable to the first 4 months of therapy.[22] As with the study in patients with head and neck cancer, an imbalance of risk factors was noted in the study reported by Leyland-Jones; epoetin-treated patients had lower performance status, more advanced disease, and more thrombosis risk factors.[22] It is also important to note that both studies reporting a negative effect of ESA treatment on survival treated non-anaemic patients pre-emptively before chemotherapy or radiotherapy. Significantly, another study in 1143 patients with breast cancer reported no effect of epoetin on disease-free or overall survival(Table 3).[83]

Further reassurance is provided by almost two decades of use in the nephrology setting.[89] Long-term ESA treatment is frequent in patients with anaemia due to renal failure, and yet no concerns have emerged with regard to possible effects on the risk of tumour development or tumour progression.

4. Conclusion

Expression of Epo-R by cancer cells, the growth-regulatory potential of Epo, and its influence on hypoxia, radiotherapy, and chemotherapy are areas of active preclinical and clinical research. These studies are scientifically important, and potentially valuable for delineating signalling pathways and exploiting the potential pleiotropic effects of Epo. However, the diverging results in the literature and methodological limitations in many experiments indicate that findings from preclinical studies must not be over-translated in terms of their clinical relevance. Clinical decisions on the use of ESA treatment should be based on evidence from clinical studies.

Conflict of interest statement

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