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Hypoxia and Aggressive Tumor Phenotype: Implications for Therapy and Prognosis

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ABSTRACT

Tumor hypoxia, mostly resulting from poor perfusion and anemia, is one of the key factors in inducing the development of cell clones with an aggressive and treatment-resistant phenotype that leads to rapid progression and poor prognosis. Studies in patients with solid tumors suggest that there is a range of hemoglobin (Hb) concentrations that is optimum for tumor oxygenation. When used to achieve an Hb level within this range, erythropoiesis-stimulating agents (ESAs) can be expected to increase tumor oxygenation, and this may favorably influence sensitivity to treatment as well as quality of life. There is no robust evidence that ESAs, when used as indicated, have a negative effect on survival in patients with solid tumors. When used outside the indications recommended, the rise in Hb level that results may reduce tumor blood flow and tissue oxygenation because of a raised viscosity within the abnormal tumor microvasculature. In the current situation, it remains important to use ESAs within the approved indications and according to treatment guidelines such as those developed by the European Organization for Research and Treatment of Cancer. ***The Oncologist*** 2008;13(suppl 3):21–26

INTRODUCTION

For many years, and perhaps among radiation oncologists in particular, it has been widely considered that improving tumor oxygenation is likely to increase sensitivity to treatment. In this context, erythropoiesis-stimulating agents (ESAs) were thought to have a potentially valuable role. Recent concern that ESAs, albeit when used for purposes that are not currently indicated, may adversely affect survival has led us to review their role [1, 2]. It is therefore helpful to look once again at the relationship between tumor oxygenation and pathophysiology. It appears that, while ESAs can have a valuable role in correcting anemia within the indicated range, their use to increase hemoglobin (Hb) levels beyond the recommended target of 12 g/dl has no further benefit and may indeed have adverse effects on tumor oxygenation and response to therapy. In this context, more is not necessarily better.

Hb LEVEL AND OXYGENATION IN SOLID TUMORS

Several groups have investigated the relationship between tissue oxygen tension distributions in solid tumors and Hb concentration. In cancers of the head and neck prior to treatment, Becker and colleagues found that the median partial pressure of oxygen (pO_2) in the tumor rose as the Hb concentration increased from 10 to 14 g/dl, but then fell once the Hb level reached 15 g/dl (Fig. 1A) [3]. We found a similar relationship in patients with squamous cancers of the uterine cervix: tumor pO_2 peaked at an Hb level of 12–13 g/dl and then declined at concentrations above this value (Fig. 1B) [4].

These findings were surprising given what is known about the relationship between oxygenation status and Hb concentration in healthy tissues. In the normal breast, the median pO_2 remains relatively constant over the range of Hb levels of 8–15 g/dl [5]. In breast cancer, however, the median pO_2 falls

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steadily as Hb decreases over this range (Fig. 2) [5]. In malignant tissue, pO_2 is consistently lower than that in healthy tissue at all Hb values, and tumors are invariably hypoxic, with a median pO_2 of <10 mmHg when Hb is <13 g/dl.

It is likely that several factors contribute to tumor hypoxia. The abnormal structure and function of the tumor microvasculature will reduce perfusion, increase the amount of fluid leaking into the extravascular space of the tumor, and thus increase the viscous resistance to flow. Reduced tumor blood flow increases the hypoxic tissue fraction, especially at lower Hb concentrations. Thus, anemia arising as a result of the malignant process itself or of anticancer treatment will play a pivotal part in worsening tumor oxygenation.

The relationship between Hb level and pO_2 assumes importance given the evidence that tumor hypoxia is an independent prognostic factor associated with poor survival. This has been demonstrated, for example, in patients treated for cervical cancer [6]. Among 48 patients whose median pO_2 was <10 mmHg, <40% were alive at 3 years; among 89 patients with a higher median pO_2, the survival rate at 3 years approached 80% (Fig. 3) [6].

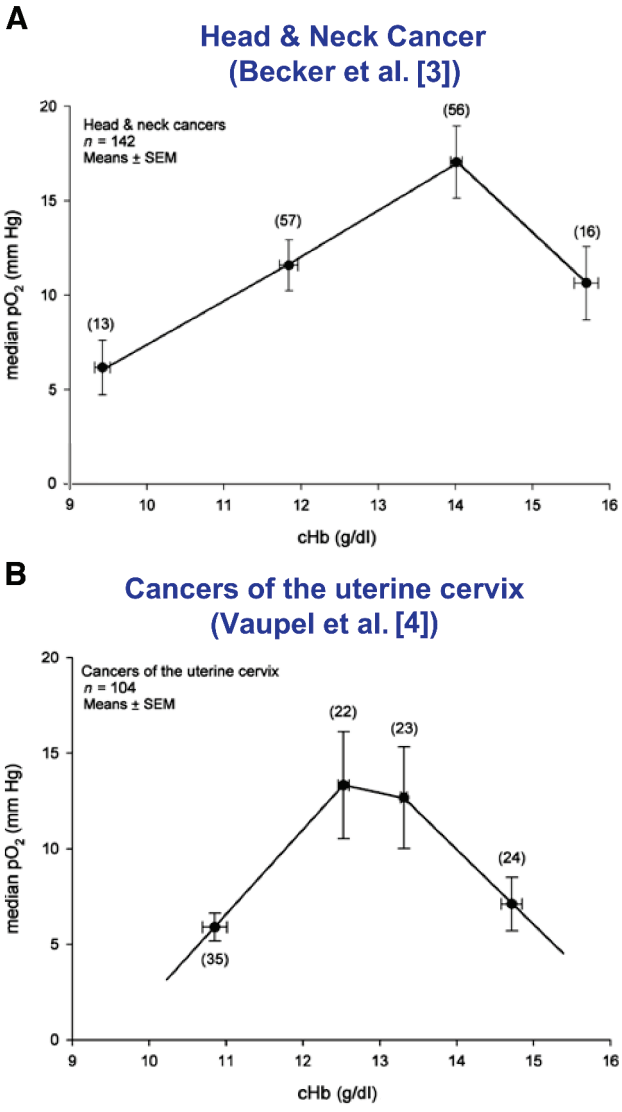


Figure 1. Oxygenation status as a function of Hb level: Clinical investigations. Shown are results of studies showing that there is an optimal oxygenation status within a gender-specific Hb range. From Vaupel P, Mayer A, Höckel M. Impact of hemoglobin levels on tumor oxygenation: The higher, the better? Strahlenther Onkol 2006;182:63–71, with permission.
Abbreviations: cHb, hemoglobin level; pO_2, partial pressure of oxygen; SEM, standard error of the mean.

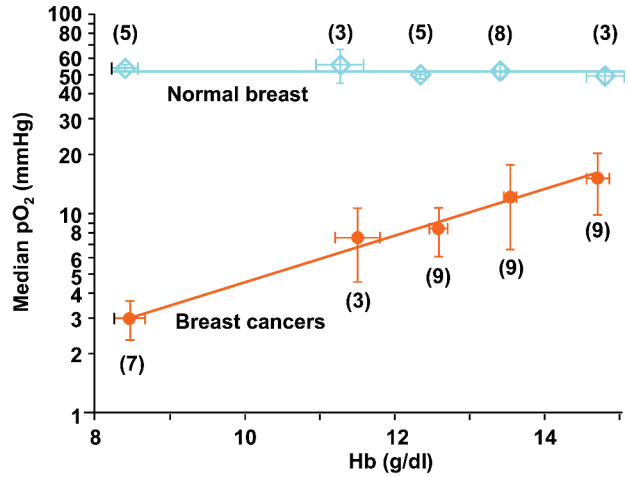


Figure 2. Oxygenation status as a function of Hb level. From Vaupel P, Mayer A, Briest S et al. Oxygenation gain factor: A novel parameter characterizing the association between hemoglobin level and the oxygenation status of breast cancers. Cancer Res 2003;63:7634–7637, with permission.
Abbreviations: Hb, hemoglobin concentration; pO_2, partial pressure of oxygen.

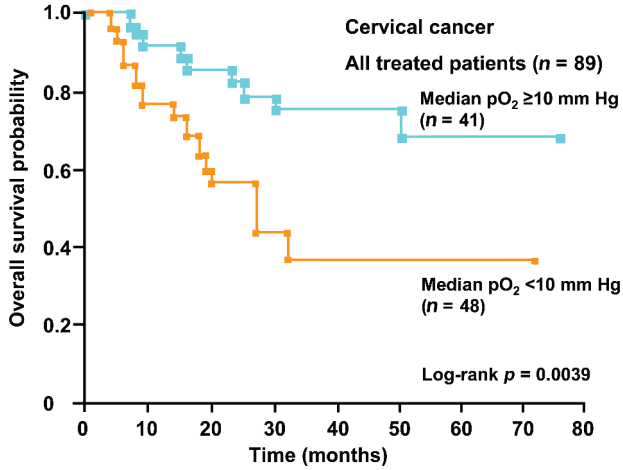


Figure 3. Tumor oxygenation and survival. From Höckel M, Schlenger K, Aral B et al. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56:4509–4515, with permission.
Abbreviations: pO_2, partial pressure of oxygen.

Hypoxia-Induced Changes

What Happens at Tissue O_2 Concentrations <1% (pO_2 <7 mmHg)?

Tumor hypoxia has two sides to it (Fig. 4A) [7]. On the one hand, tissue O_2 concentrations <1% (pO_2 <7 mmHg) can exert antiproliferative effects: They can restrict cell proliferation, may promote differentiation, and may induce apoptosis and necrosis (Fig. 4A, B) [8]. On the other hand, there are clones within the tumor that react to hypoxic stress with adaptive processes that, through modification of gene expression, confer an aggressive phenotype, promoting local and distant spread (Fig. 4A, B) [8–12].

Hypoxia also promotes angiogenesis, which involves the formation of new blood vessels from pre-existing blood vessels [13, 14]. Generally, tumors are unable to grow beyond a diameter of approximately 1 mm because of an inadequate microenvironment and the resultant lack of essential nutrients and O_2 [14]. For the tumor to increase in diameter, the vascularization must correspondingly increase [13]. Inadequate O_2 supply can result in hypoxia (pO_2 <7 mmHg), which can stimulate angiogenesis through various growth factors [14–17]. Growth factors such as vascular endothelial growth factor, platelet-derived growth factor-B, transforming growth factor β, insulin-like growth factor-2, and epidermal growth factor are upregulated, whereas angiogenesis inhibitors (i.e., angiostatin, endostatin, 16 kDa protactin, leukemia inhibitory factor) can be shut down [13, 14, 18–22]. However, the vessels formed in this process are not the same as those found in normal tissue. These newly formed tumor blood vessels are irregularly shaped, have gaps between endothelial cells allowing leakiness, have an incomplete hierarchical structure, and have functional abnormalities [14, 15, 23, 24]. Antiangiogenic drugs may add benefit to cancer treatment, most probably by "normalizing" tumor vasculature.

Many of the changes observed in hypoxia are regulated by hypoxia-inducible factor 1 (HIF-1) (Fig. 4B) [14, 25]. HIF-1α is a transcription factor that regulates the expres-

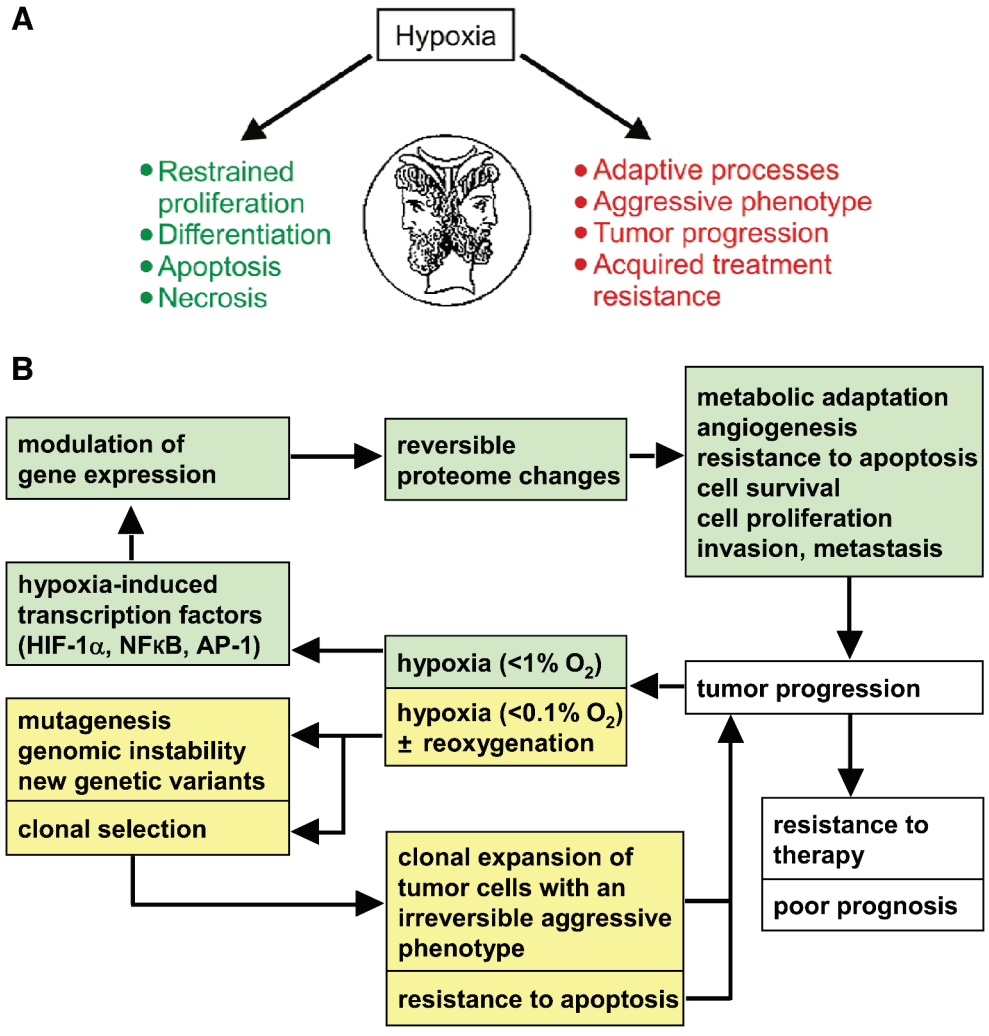


Figure 4. The "Janus face" of tumor hypoxia **(A)** and mechanisms causing malignant progression and treatment resistance **(B)**. **(A):** From Vaupel P, Mayer A. Effects of anaemia and hypoxia on tumor biology. In: Bokemeyer C, Ludwig H, eds. Anaemia in Cancer. European School of Oncology Scientific Updates, Second Edition: 47–54. ©Elsevier 2005. Reprinted with permission from Elsevier.
Abbreviations: AP-1, activator protein 1; HIF-1α, hypoxia inducible factor 1α; NFκB, nuclear factor κB.

sion of >30 target genes involved in hypoxia-induced erythropoietin expression, tumor progression, and tumor aggressiveness [14, 26, 27]. Again, these changes in gene expression and proteomic changes occur at O_2 levels <1%, or <7 mmHg. Hypoxia may also facilitate metastasis, through downregulation of adhesion molecules [14, 28]. The tumor becomes rapidly progressive and acquires resistance to treatment with either chemo- or radiotherapy.

What Happens at Tissue O_2 Concentrations <0.1% (pO_2 <0.7 mmHg)?

Severe hypoxia induces genomic instability and increased selection pressure (Fig. 5A) [14]. Genomic changes and clonal selection occur at O_2 levels <0.1%, or <0.7 mmHg (Fig. 5B) [14, 29]. The aggressive phenotype that results can be considered as a desperate attempt of cancer cells to survive the hostile and nutrient-deprived microenvironment that leads to faster tumor progression that further enhances hypoxia and thus perpetuates this vicious circle.

While the great majority of tumor cells suffer adverse consequences under hypoxic conditions, a small fraction (perhaps <5%) behave quite differently, and selection pressure may favor this minority of cells that can determine the fate of the patient.

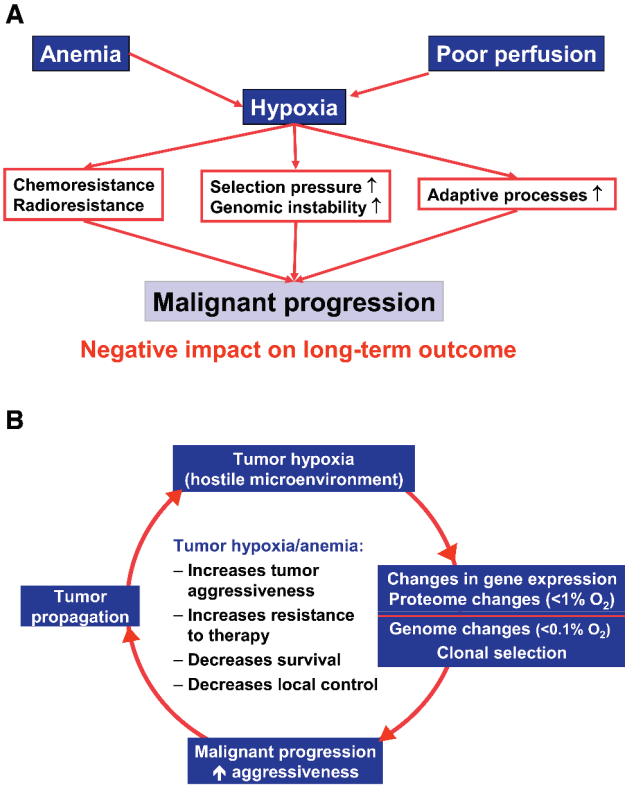


Figure 5. Hypoxia-induced changes in genome and protein expression leading to increased aggressiveness of the disease, with malignant progression [14, 29]. **(A):** Hypoxia-induced mechanisms leading to selection of aggressive tumor cell phenotypes and resistance to therapy. **(B):** Vicious circle of tumor hypoxia and malignant progression.

Anemic Patients

In anemic patients, hypoxia is more pronounced [30]. Increases in the Hb level can be expected to have a favorable effect on prognosis only when they result in better tumor oxygenation. This is unlikely to be the case when Hb concentrations rise to >14 g/dl. At this level, it is anticipated that the rise in viscosity and resistance to flow within the chaotic microvasculature of the tumor will lead to a net reduction in O_2 supply.

Implications for the Use of ESAs

It is in this context that the use of ESAs must be considered. In a study by Henke et al. [1], which has attracted considerable attention, epoetin beta was used off-label in head and neck cancer patients being treated with radiation, with the aim of achieving radiosensitization by increasing Hb levels to 14 g/dl in women and 15 g/dl in men. These targets were achieved in 82% of patients treated with epoetin and in 15% of controls (Fig. 6A) [1]. However, there was also a negative impact on progression-free and overall survival times. The likely reason is that the starting level of Hb was inappropriately high (median baseline values were 11.7 g/dl in the epoetin group and 11.8 g/dl among placebo patients), as was the ultimate target of ESA therapy. Against this background, the use of epoetin can be regarded as overtreatment that resulted in Hb levels beyond the optimum range for tumor oxygenation suggested by the work (mentioned above) of Becker et al. [3] and Vaupel et al. [4] (Fig. 6B) [1, 3, 4, 31]. Many placebo patients, in contrast, have had Hb levels that were within the optimum range.

In the case of the BEST study in breast cancer (Breast Cancer Erythropoietin Survival Trial), treatment with epoetin was begun if the Hb level was ≤13 g/dl at baseline [2]. It is likely that the poorer survival seen in this trial among patients given the ESA was again related to the high Hb level that resulted. Such off-label studies should not undermine the use of ESAs within the clearly defined clinical settings in which trials have demonstrated their benefit. A value of 12 g/dl is an appropriate Hb concentration to use as the target of ESA therapy, not as its starting point. The range 12–14 g/dl can perhaps represent the upper range that may still be tolerated. Levels >14 g/dl should be avoided.

It should also be noted that the alternative to the use of ESAs, that is, blood transfusions, leads to large fluctuations in Hb level in which the troughs clearly lie below the optimum range for tumor oxygenation (Fig. 7A) [32]. This

A

Hb increases
Henke et al. (2003) [1]

Mean Hb level (g/dl)	Epoetin beta (*n* = 180)	Placebo (*n* = 171)
Baseline	11.7	11.8
4 weeks	14.8	12.4
9 weeks	15.4	12.9

B

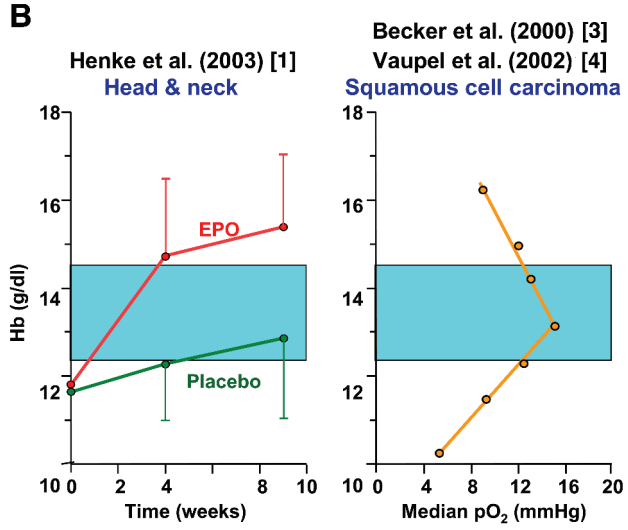
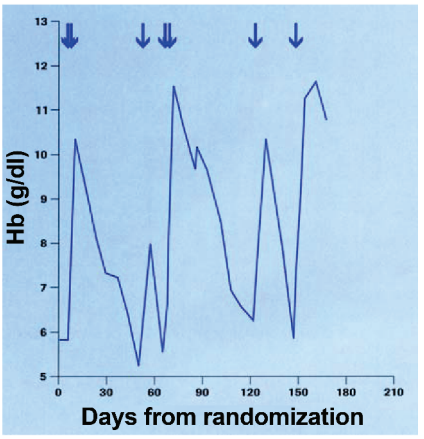


Figure 6. Hb level and oxygenation status in head and neck cancers [1, 3, 4, 31]. **(A):** Head and neck cancer patients were randomized to epoetin beta (300 IU/kg s.c. three times a week) plus radiation therapy or placebo plus radiation therapy: Hb concentration increased over time. The study target Hb of ≥14.0 g/dl for women and ≥15.0 g/dl for men was achieved by 82% of the epoetin beta group ($n = 148$) and 15% of the placebo group ($n = 26$). **(B):** Maximum tumor oxygenation was found over the Hb range of 12.2–14.4 g/dl. The Henke et al. (2003) [1] study was beyond this level. From Vaupel P, Dunst J, Engert A et al. Effect of recombinant human erythropoietin (rHuEPO) on tumor control in patients with cancer-induced anemia. Onkologie 2005;28:216–221, with permission.
Abbreviations: Hb, hemoglobin level; pO_2, partial pressure of oxygen.

A



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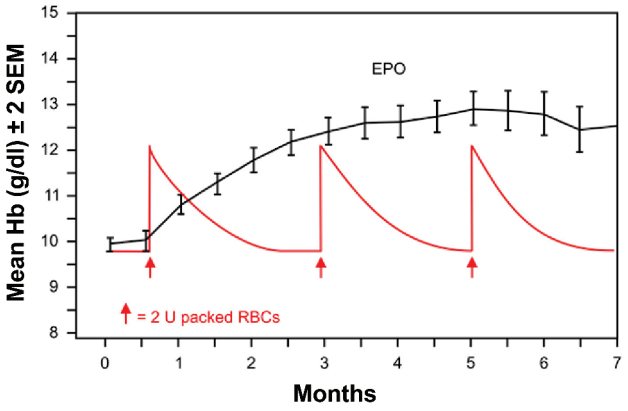

Figure 7. Fluctuations in Hb concentrations after repeated RBC transfusions. **(A):** Arrows indicate repeated allogeneic RBC (500 ml) transfusions [32]. From Österborg A. Recombinant human erythropoietin (rHuEPO) therapy in patients with cancer-related anaemia: What have we learned? Med Oncol 1998;15(suppl 1):S47–S49, with kind permission of Springer Science and Business Media. **(B):** EPO administration maintained the mean Hb concentration at an optimal level [33], without the Hb concentration drops observed between RBC transfusions (schematic representation).
Abbreviations: EPO, erythropoietin; Hb, hemoglobin level; SEM, standard error of the mean.

contrasts with the use of ESAs that achieve, through steady increments, an Hb level consistently within the optimum range (Fig. 7B) [33]. Cyclic hypoxia, of the kind that results from repeated RBC transfusions, is also believed to strongly promote an aggressive tumor phenotype [34–36].

Conclusion

Only increases in Hb levels that result in improvements in tumor oxygenation can be expected to have a favorable effect on prognosis. At higher Hb levels (>14 g/dl), a substantial rise in the viscous resistance to flow within the chaotic tumor microvasculature can be expected and may lead to a net reduction in O_2 supply, and thus poor tumor oxygenation. Only increases up to the currently recommended target Hb levels result in improvements in tumor oxygenation, and thus may improve prognosis. ESAs should be used as indicated and within the current European Organization for Research and Treatment of Cancer guidelines to achieve the optimum Hb levels [37].

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