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SERIES EDITORS:
U. VERONESI & M.S. AAPRO

Anaemia in Cancer

SECOND EDITION

EDITED BY C. BOKEMEYER AND H. LUDWIG

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EUROPEAN SCHOOL OF ONCOLOGY SCIENTIFIC UPDATES, VOLUME 6
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Anaemia in Cancer

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Foreword

The European School of Oncology (ESO) is a non-governmental, non-profit-making organisation, which was founded in 1982. It has since become a model for many other professional groups that need to provide timely information, education and training. The rate of progress in the areas of diagnosis and treatment of cancer is so rapid that oncologists need continuous updating in order to provide their patients with the best chances for palliation or cure. ESO recognises the multidisciplinary nature of cancer treatment, and its activities encompass all medical, nursing and technical specialities dealing with neoplastic diseases. Timeliness and scientific accuracy are the key factors behind the numerous activities of the School.

The *Scientific Updates* are a series of books designed to disseminate the full results of topical meetings organised by the School, often after preparatory work has been performed by a task force. Each issue is under the responsibility of one or several volume editors, and contains the latest information on a particular subject area. To preserve the timeliness of the information, the ESO *Scientific Updates* utilise a simple layout and presentation to enable very rapid publication times, thus overcoming a common problem in the medical literature: that of the material being outdated even before publication.

The editors welcome suggestions for further issues and, together with the whole ESO staff, are ready to help interested parties in establishing new task forces and subsequent meetings. In this way the knowledge of new developments in all fields of oncology, from basic research through to nursing, can be shared. Education and training are important in helping reduce cancer-related mortality, and the ESO is firmly committed to achieving this aim.

Matti S. Aapro
ESO Scientific Updates
Series Editor

Contents

ESO Scientific Updates, Vol. 6, 2nd Edition
Anaemia in Cancer
C. Bokemeyer and H. Ludwig, editors

Introduction

Carsten Bokemeyer and Heinz Ludwig

This book presents a comprehensive overview of the pathogenesis, frequency, impact and treatment of chronic anaemia of cancer as well as radio- and chemotherapy-induced anaemia, according to the state of the art in 2004. It is the result of a joint effort by the members of the ESO Task Force on Anaemia in Cancer and an outstanding international faculty of recent ESO and EORTC symposia dedicated to this topic, which were held in Milan and Rome between 2001 and 2004. This important educational activity was made possible by an unrestricted educational grant from Amgen (Europe) GmbH.

Anaemia, a frequent complication of cancer, is often associated with clinical sequelae that are frequently underestimated or not recognised at all. Anaemia in cancer patients is now also considered as a significant cause of overall reduced quality of life. During the last decade considerable insight has been gained into the pathogenesis of anaemia in cancer and the role of chemo- and radiotherapy-induced anaemia, a complication that may aggravate pre-existing chronic anaemia of cancer.

Anaemia has also been identified as a negative prognostic factor for clinical outcome in cancer patients. In addition, there is evidence that tissue hypoxia contributes to the progression of malignant disease by inducing a more malignant phenotype, stimulating angiogenesis within tumour tissues, and diminishing the sensitivity of tumour cells to chemotherapy and radiation therapy.

These problems have long remained unchallenged due to the fact that the traditional treatment for anaemia, i.e. the transfusion of red blood cells, has significant limitations, not only because of the inherent risks of infections, immunological reactions, iron and volume overload, but mainly because of the treatment's inability to bring about long-lasting and complete correction of anaemia. This situation has changed dramatically with the introduction of recombinant human erythropoietin, which acts as an important physiological stimulator of erythropoiesis. The beneficial effects of erythropoietic growth factors exceed those of transfusions. Erythropoietic growth factors can lead to a complete, long-term normalisation of anaemia, which, in turn, results in a significant improvement in anaemic cancer patients' quality of life and physical exercise capacity. Darbepoetin-alpha has become available as a novel treatment for anaemia in addition to rHuEPO in recent years. This novel erythropoietic stimulating molecule has been approved for the treatment of chemotherapy-

induced anaemia in patients with non-myeloid malignancies. Its prolonged half-life allows once weekly administrations and extended schedules of one s.c. injection every 2, 3 or 4 weeks are in development. Furthermore, the increased biological activity of darbepoetin-alpha is being investigated using upfront escalated doses to achieve a more rapid correction of anaemia and a higher number of responders than with conventional treatment schedules.

While preliminary studies had indicated that erythropoietin treatment may also improve the outcome of cancer therapy in specific situations, this question has meanwhile been addressed in randomised trials. Despite the initial optimism, caution is necessary when using erythropoietic growth factors in this setting and it appears that there will be no easy "yes" or "no" answer to the question on the impact of erythropoietic growth factors on survival.

In fact, two randomised studies using erythropoietic agents outside of the presently approved indications have reported a worse outcome in patients receiving the agents. It should thus be emphasised that the use of erythropoietic agents should follow the international and national guidelines and respect the product labels.

In summary, the rapidly expanding knowledge of anaemia and its treatment in cancer patients has made a second edition of this ESO monograph necessary, only 3 years after the first edition. The topics addressed in this book are both of high scientific interest and of practical value for physicians caring for cancer patients.

ESO Scientific Updates, Vol. 6, 2nd Edition
Anaemia in Cancer
C. Bokemeyer and H. Ludwig, editors

Erythropoiesis and Anaemia

Photis Beris

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Introduction

Healthy individuals require an adequate production of enormous numbers of differentiated blood cells daily. Thus, as many as 1–5 $\times$ 10^9 erythrocytes and 1–5 $\times$ 10^9 white blood cells are produced per hour during the lifetime of an individual. This haematopoietic process can be separated into four different functional compartments:

1. Stem cells
2. Progenitor cells
3. Precursor cells
4. Mature cells

The *stem cell* compartment comprises rare primitive cells that are *multipotential* (i.e., they can develop into all lineages of blood cells) and have a great self-renewal capacity (i.e., they give rise to identical daughter stem cells). Most of the stem cells in this compartment are mitotically quiescent. *Progenitor cells* are characterised by commitment to differentiation into one or more lineages and possess limited self-renewal capacity. Under the microscope, *precursor cells* exhibit easily recognisable nuclear and cytoplasmic morphological characteristics that can be used to classify the lineage to which they belong. These cells have a marked mitotic activity and constitute the great majority of cells in the bone marrow environment. The *mature red cell* (erythrocyte) is the final phase of a complex but orderly series of genetic events that begins at the time a multipotent stem cell commits to the erythroid programme. Erythrocytes have a finite life span (~120 days), and when they leave the bone marrow microenvironment, they have an immature but anuclear form; in this stage they are called reticulocytes.

During the past decade, many mechanisms governing erythropoiesis have been elucidated and complex interactions of erythroid progenitors and precursor cells with the bone marrow microenvironment have been discovered. These

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findings have allowed a better understanding of the physiology of erythropoiesis and the pathophysiology of various clinical disorders characterised by failure or neoplastic transformation of the erythroid tissue. As a direct consequence of this, the basis of new therapies for different forms of anaemia has been created. In this chapter we will review the current concepts of normal erythropoiesis and discuss a comprehensive classification of the anaemias.

Erythropoiesis

Ontogeny of human erythropoiesis

Haematopoietic stem cells (HSCs) are found in the aorta–gonadal–mesonephros (AGM) and probably arise from haemangioblastic cells that can differentiate into both HSCs and endothelial cells [1]. HSCs also originate from the extra-embryonic mesoderm in the yolk sac [2]. After 5 weeks' gestation, HSCs colonise the liver, from where they migrate to the bone marrow and thymus at 8 weeks and to the spleen at 12 weeks.

Another hypothesis is that HSCs from the AGM region first colonise the yolk sac and then the liver and bone marrow. According to this hypothesis, haematopoiesis derives from the same stem cell pool [3]. In the fetal liver, haematopoiesis is predominantly erythroid, while in the fetal bone marrow it is mainly myeloid. Lymphopoiesis in the lymph nodes starts approximately at week 20. After birth, the bone marrow becomes the main haematopoietic organ.

It should be noted that, during fetal life and immediately after birth, a large number of stem cells are found in the circulation. This has led to the use of cord blood as a source of stem cells in the treatment of haematological malignancies, congenital immune deficiencies and certain hereditary haemoglobinopathies [4]. Figure 1 is a graphical representation of human haematopoiesis depicting primitive (embryonic) and definitive (fetal and adult) haematopoiesis.

The reasons for the migratory behaviour of HSCs and the mechanisms underlying their localisation, migration and homing to different organs throughout embryonic and fetal life are not completely understood. Changes in the adhesive behaviour of HSCs at different stages of ontogenic differentiation,

Table 1. Haematopoiesis

Primitive (embryonic)	**Definitive (fetal → adult)**
yolk sac	fetal liver → bone marrow
begins ~ day 15	begins ~ day 35
only nucleated erythrocytes	non-nucleated erythrocytes
ζ and ε globin chains	α, γ and β globin chains

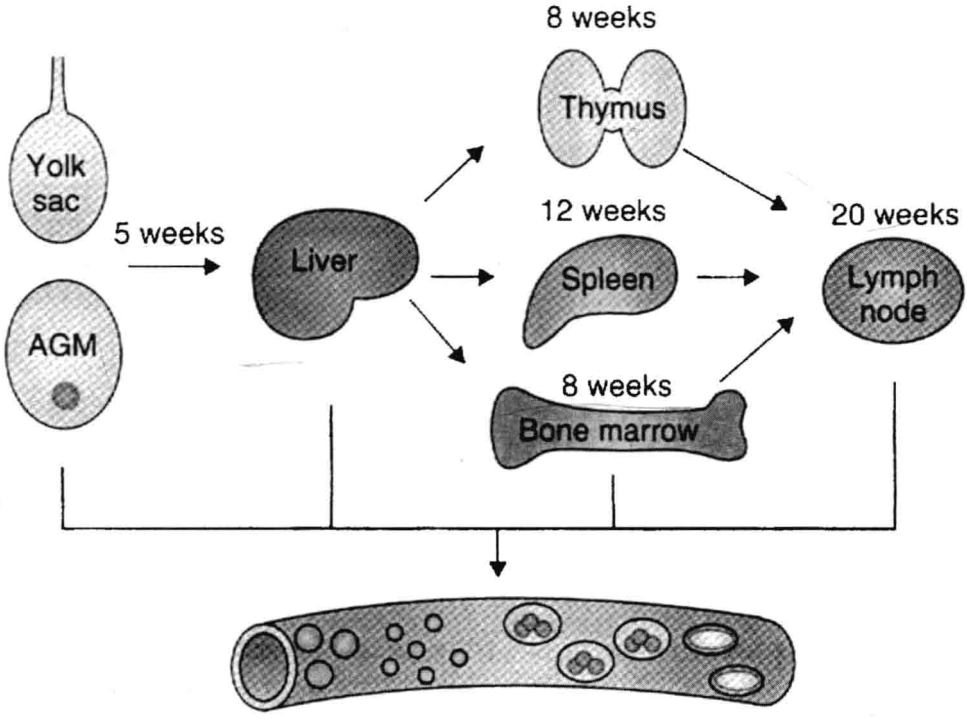

Fig. 1. Ontogeny of human haemopoiesis. AGM, aorta-gonadal-mesonephros. Reproduced from Verfaillie CM. Anatomy and physiology of hematopoiesis. In: Hoffman R, Benz EJ, Shattil ST et al., eds. Hematology, Basic Principles and Practice, 3rd ed. Philadelphia: Churchill Livingstone 2000; 139–54 with permission from Elsevier.

or differences in the capacity of the different haematopoietic organs to support the development of progenitors of different ontogenic ages, may underlie the sequential establishment of haematopoiesis in AGM, yolk sac, liver, spleen and bone marrow [5].

Erythroid progenitor and precursor cell compartment

Erythroid progenitor cells are sparse and consist of two classes, the burst-forming unit erythroids (BFU-E), so-called on account of their ability to give rise to multiclustered colonies (erythroid bursts) of haemoglobin-containing cells after 14–16 days, and the colony-forming unit erythroids (CFU-E), which generate small colonies within 7 days [6]. BFU-Es have physical and functional properties that are shared by pluripotent stem cells. In contrast, CFU-Es have many similarities to erythroid precursors: their proliferative potential is limited, they cannot self-renew, and they are exquisitely sensitive to erythropoietin (EPO).

Table 2. Erythroid progenitors: surface antigens/receptors

	BFU-E	CFU-E
CD34	++	–
CD33	+	0
C-kit	++	+
HLA DR	++	+
EPO-R	+	++
TNF-R	+	++
Gly A	0	+
ABH, iI	+	+

HLA DR, human leukocyte antigen DR; EPO-R, erythropoietin receptor; TNF-R, tumour necrosis factor receptor; Gly A, glycophorin A; ABH, iI, blood groups A, B, H, i, I; ++, present in high concentrations; +, present; –, rare; 0, absent.

Table 2 defines BFU-E and CFU-E in relation to the presence of surface antigens/receptors.

A number of cytokines have been shown to influence the proliferation and/or survival of erythroid progenitors: Kit-ligand, IL-3, GM-CSF, IL-11 and thrombopoietin have a profound proliferative effect on BFU-Es and their progeny. Conversely, TNF-α, transforming growth factor-β and interferon-γ negatively influence BFU-E proliferation.

The erythroid precursor cell compartment, known also as the erythron, includes cells that are defined by morphological criteria. The main steps of erythroid maturation are shown in Figure 2.

The proerythroblast is the earliest recognisable erythroid precursor. The nuclear/cytoplasmic ratio is high, chromatin is fine, and one or two nucleoli are seen. The cytoplasm is basophilic, containing almost no haemoglobin; the Golgi apparatus is well developed. After 4–5 mitotic divisions, basophilic erythroblasts are formed, followed by polychromatophilic and orthochromatic or oxyphilic erythroblasts. The morphological characteristics of the cytoplasm reflect the accumulation of haemoglobin. There are many transferrin receptors on the erythroid precursors and fewer on the reticulocytes, which are the earliest erythroid anuclear cells.

Figure 3 is an electronic microphotograph showing the polar position of the nucleus of the orthochromatic erythroblast, which is subsequently extruded and encased by a thin cytoplasmic layer. The loss of vimentin expression during the last erythroblastic stages most probably facilitates enucleation [7].

Gene activity during erythroid maturation is dominated by the expression of globin, erythroid-specific enzymes of the haeme metabolic pathway, and membrane cytoskeletal proteins (spectrin, glycophorin, band 3, band 4.1, ankyrin).

Compared to erythroid progenitor cells, erythroid precursor cells have been more accessible to study and a considerable amount of information is available

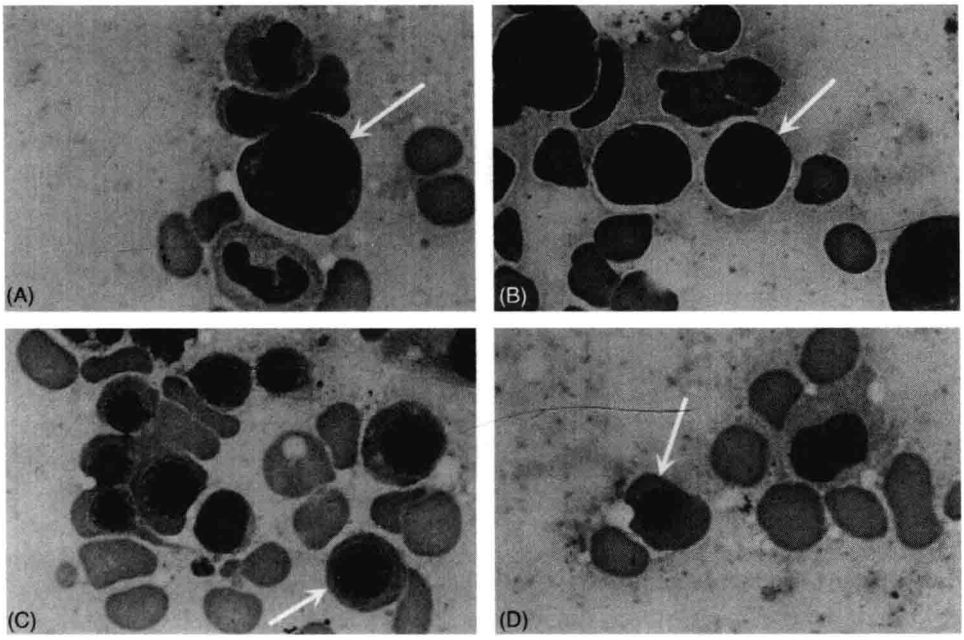

Fig. 2. Main maturation steps of erythroid precursors. A. Proerythroblast. Note the well-developed Golgi apparatus. B. Basophilic erythroblasts. Cytoplasm is still basophilic; however, no nucleoli are seen and the cell size is smaller than that of proerythroblasts. C. Polychromatophilic erythroblasts. D. Orthochromatic or oxyphilic erythroblasts. The morphological characteristics of the cytoplasm reflect the accumulation of haemoglobin. (Bone marrow, Wright staining, original magnification ×1000.)

on their maturation-related biochemical changes. However, a detailed description of all these phenomena is beyond the scope of this chapter.

Transcriptional control of erythropoiesis

Expression of erythroid-specific genes within erythroid progenitors and precursors is mediated by the combinatorial activity of ubiquitously-expressed and tissue-restricted (i.e., erythroid-restricted) transcription factors. These factors play key roles in the regulation of gene expression by combining with DNA to coordinate the synthesis of mRNA levels for essentially all known erythroid genes. They may operate alone or as part of transcriptional complexes and induce activation or repression.

Furthermore, taking into consideration the existence of distinct phases and anatomic sites of erythropoiesis (and haematopoiesis) during development – primitive erythropoiesis in the yolk sac and definitive erythropoiesis in the fetal liver and the bone marrow (Table 1) – specific sets of regulatory proteins are

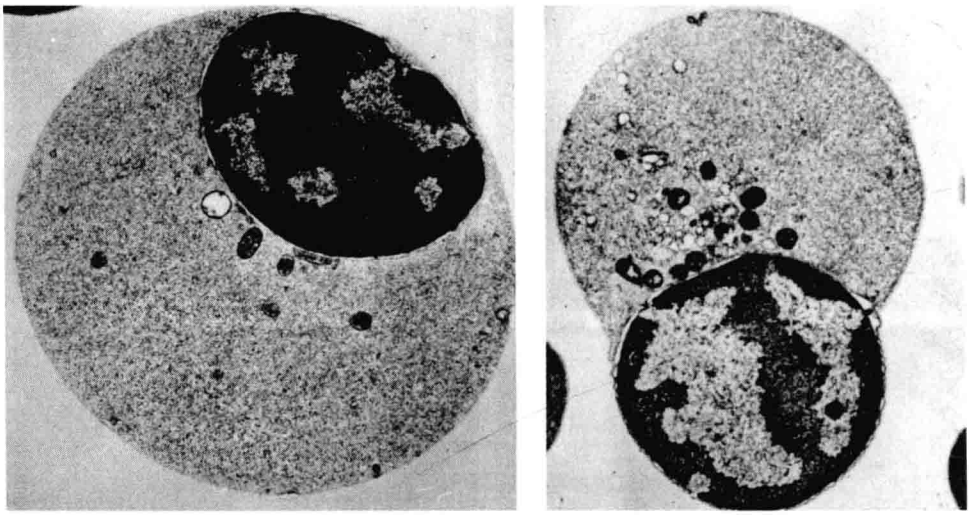

Fig. 3. Electronic microphotograph showing the genesis of a reticulocyte (see text for further details, original magnification ×20,000).

required to coordinate and ensure the ontogeny of erythroid cells and the other blood elements in general.

The basic helix–loop–helix (bHLH) factor tal-1/SCL has a critical function at a very early stage of haematopoietic differentiation. In fact, mice homozygous for deletion of the tal-1/SCL gene die *in utero* as a result of the absence of blood formation, even within the yolk sac [8]. Furthermore, *in vitro* colony assays and other studies showed that this gene is also required for myelopoiesis as well as for vascular differentiation [9].

The rbtn2/LMO2 is also required at a very early stage of haematopoiesis but continues to be expressed in definitive erythroid cells [10]. rbtn2-deficient mice die early *in utero* with a dramatic erythropoietic defect similar to that found in tal-1-deficient embryos [11]. There is evidence that rbtn2/LMO2 interacts with SCL and that both function in a common pathway of gene regulation.

The zinc finger GATA-1 is a key regulator of erythroid differentiation. The DNA sequence motif GATA is present in important *cis*-regulatory elements of many erythroid-expressed genes [12]. Targeted disruption of the GATA-1 gene results in maturation arrest of erythroid precursors at the proerythroblast stage. It may also play a role in lineage selection [13].

GATA is also expressed on megakaryocytes and on eosinophilic precursor cells. Several proteins have been reported to interact physically with GATA-1: FOG-1, LMO-2, EKLF and PU.1 [14]. Of these, FOG-1 (friend of GATA) has been the most studied, since it plays an essential role in erythropoiesis and megakaryopoiesis [15]. Mutated GATA-1 that is unable to interact with FOG fails to support terminal erythroid maturation.

Familial X-linked dyserythropoietic anaemia, due to a substitution of methionine for valine at residue 205 of GATA-1, interrupts the GATA-1:FOG-1 interaction and inhibits the ability of GATA-1 to rescue erythroid differentiation in a GATA-1-deficient erythroid cell line. This results in severe fetal anaemia and anaemia with severe thrombocytopenia at birth and thereafter, as well as cryptorchidism in the male offspring.

The substitution Ser 208→Gly or Gly 218→Asp in GATA-1 has been reported in families with recessive X-linked thrombocytopenia and X-linked macrothrombocytopenia, respectively. Again, the replaced residues are involved in GATA-1:FOG-1 direct interactions [16–18].

Haematopoietic expression of GATA-2 overlaps with that of GATA-1. Mice lacking GATA-2 exhibit a severe and early haematopoietic defect. They succumb to anaemia due to a marked reduction in the number of embryonic RBCs [19]. These results indicate that GATA-2 is essential for appropriate expansion of early haematopoietic cells. Both GATA-1 and GATA-2 are regulated in a cell type-specific manner, their expression being strictly controlled during the development and differentiation of erythroid cells. Closer examination revealed a cross-regulatory mechanism by which GATA-1 can control the expression of GATA-2 and vice versa, possibly via essential GATA bracing sites in their *cis*-lacking elements [20].

Table 3 summarises the other transcription factors involved in erythropoiesis. It is interesting to note that mice lacking EKLF die from a thalassaemia-like defect, a severe deficiency of β-globin expression [21]. Also, some patients with β-thalassaemia may carry mutations to the CACCC binding site, recognised by the EKLF transcription factor. Figure 4 shows the transcription factor requirements in haematopoiesis in relation to four different functional compartments [14].

Haematopoietic microenvironment

Within the bone marrow, haematopoiesis is restricted to the extravascular space known as the bone marrow microenvironment, which consists of three components:

1. Stromal cells, e.g., fibroblasts and endothelial cells;
2. Accessory cells, e.g., monocytes, macrophages and T cells;
3. Extracellular matrix, i.e., a protein–carbohydrate scaffold composed of collagens (types I, III, IV and V), fibronectin, laminin, thrombospondin, haemonectin, hyaluronic acid, chondroitin, dermatan and heparan sulphate.

The microenvironment provides a structure for the marrow space and a surface for cell adhesion. Furthermore, stromal and accessory cells elaborate cytokines that exert a positive regulation on haematopoiesis: GM-CSF, G-CSF, IL-1, IL-3, IL-6, IL-11, KL, flt-3 ligand and basic fibroblast growth factor. In addition, it elaborates factors that negatively influence haematopoiesis such as TGF-β, INF-γ and TNF. Adhesion of cells to the extracellular matrix is mediated by β1 integrins, VLA-4 and VLA-5, C-kit, CD31, CD44 and L-selectin [22].